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THE EFFECTS ON RESPIRATORY METABOLISM PRODUCED BY EQUAL AMOUNTS OF CAFFEINE IN THE FORM OF COFFEE, TEA AND THE PURE ALKALOID

JOHN HALDI, GEORGE BACHMANN, CHARLES ENSOR AND WINFREY WYNN

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ONE FIGURE

(Received for publication September 27, 1943)

The present experiments were undertaken to determine whether caffeine in coffee or in tea differs in its effect on the respiratory metabolism from the same amount of the pure caffeine alkaloid in solution. In a study of reaction time as influenced by caffeine, Cheney ('35) found that coffee produced results parallel to those obtained from capsulated caffeine but to a lesser degree. Large doses of caffeine always led to a decrease in the reaction time but the maximum reduction was usually greater with pure caffeine than with an equivalent amount in the form of coffee as a beverage. These results would suggest that the caffeine in coffee may be absorbed more slowly or to a lesser extent than from an alkaloid solution. If there are any differences in the rate or extent of absorption they should be reflected in the respiratory metabolism, which is definitely affected by caffeine (Haldi, Bachmann, Ensor, and Wynn, '41).

METHOD

The procedure was essentially the same as in our previous experiments ('41) on the respiratory exchange following the ingestion of caffeine alkaloid. Immediately after the usual preliminary basal period, the subject drank 200 ml. of a fresh infusion of coffee or tea at 37°C. or an equal volume of water containing caffeine alkaloid in solution. The same packages of a standard brand of coffee and of tea were used throughout the experiment. Ten grams of tea were added to 220 ml. boiling water, stirred for 1 minute and allowed to stand for 9 minutes. As much of the supernatant tea as could be recovered was poured off and made up to exactly 200 ml. Analysis of eight different infusions gave an average caffeine content of 195 mg. in 200 ml. tea with an average deviation of 6 mg. A coffee infusion made in the same

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way from 25 gm. of coffee contained an average of 204 mg. caffeine. For the caffeine alkaloid solution 200 mg. pure caffeine was dissolved in 200 ml. water. The unavoidable difference of several milligrams caffeine in the coffee and tea infusions and in the caffeine solution was regarded as of no consequence inasmuch as it had been found in the former experiments that 60 mg. caffeine had no effect on the respiratory metabolism. Two minutes after the ingestion the subject reclined on a couch and began breathing through the apparatus immediately. The gaseous exchange was determined at intervals of 15 minutes for a period of 1 hour and 45 minutes.

The subjects were well trained for this type of experiment, having taken part in several hundred other experiments of a similar nature. More reliable results, we believe, can be obtained from one or two subjects with adequate training than by taking averages of data derived from a large number of untrained subjects. As we have had occasion to observe before, errors may be introduced either by restlessness, or dozing or incomplete relaxation on the part of the subject, and in our experience, careful selection of the subjects and sufficient training are essential prerequisites for reliability of the experimental data.

Since it is possible that any effect produced on the respiratory exchange by coffee and tea might be due in part to other extractives than caffeine, a series of experiments were run as controls in which decaffeinated coffee was ingested. Analysis showed that the caffeine content was negligible. Eight experiments were done on each subject with each beverage.

RESULTS

Oxygen consumption

A dose of 0.2 gm. caffeine alkaloid had a definite stimulating action on oxidative metabolism as shown in the curves of oxygen consumption in figure 1. The rate of oxidation rose shortly after ingestion of the alkaloid and reached a peak (14.3% above the basal) during the first 15-minute period in one subject and within 30 minutes in the other (12.9% above the basal). This was followed by a gradual decline. At the conclusion of the experiment, however, the oxygen consumption had not returned to the basal level. These results are in conformity with those obtained in a previous study on caffeine (Haldi et al., '41).

Comparison of the curves in figure 1 shows that the stimulation of metabolism by coffee and tea was practically the same as that produced by the alkaloid. The small differences observed in some of the individual periods were within the normal range of variation. The total

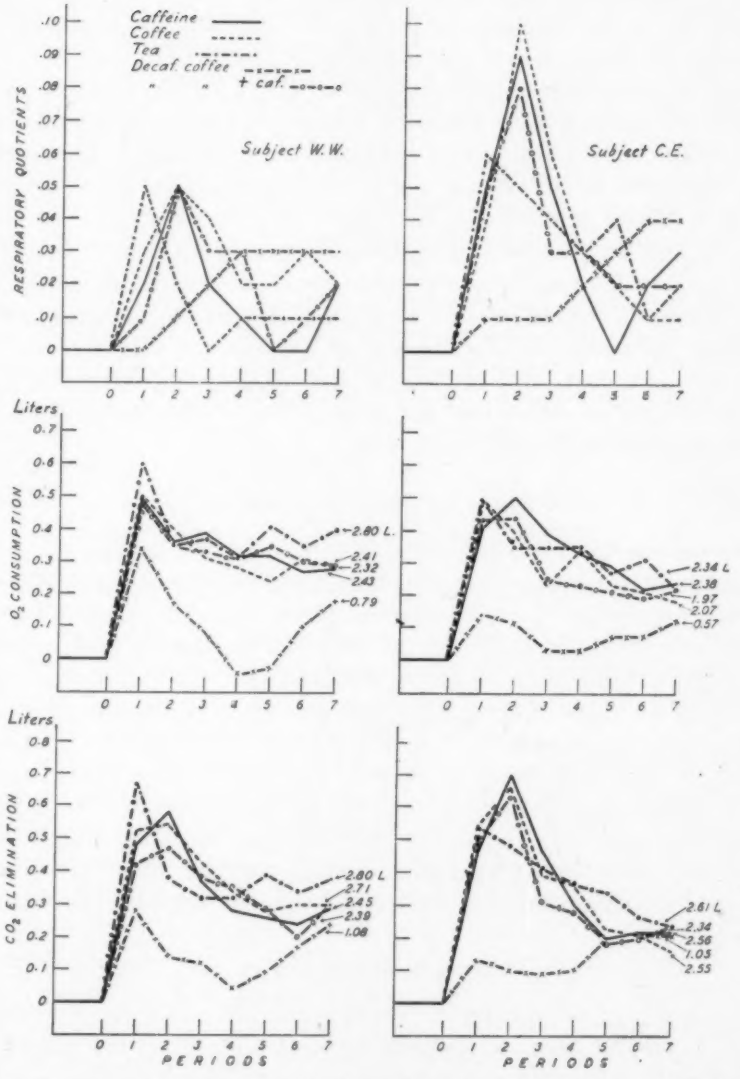

Fig. 1 The course of respiratory metabolism with reference to basal conditions following the ingestion of various beverages containing caffeine or decaffeinated coffee. The total excess metabolism above basal is indicated at the end of each curve.

increase in oxygen consumption above the basal for the entire experiment is given in the right hand margin of the curves in figure 1. The differences in the increase in oxygen consumption after taking tea, coffee, or the alkaloid were not statistically significant.

That the stimulating effect of coffee on oxidation was due to the caffeine content is evident from the experiments in which decaffeinated coffee with caffeine added was ingested. The increase in oxidation in these experiments (fig. 1) was approximately the same as that induced by coffee. These results are in agreement with those reported by Horst, Willson, and Smith ('36). The increase in oxidation after taking decaffeinated coffee was the same as has been obtained in numerous other experiments on our subjects following the ingestion of the same amount of water. Although no experiments were conducted with decaffeinated tea, it may be concluded in view of the comparative effects of coffee and decaffeinated coffee that the stimulation of oxidative metabolism by tea was due to its caffeine content.

Carbon dioxide elimination

The carbon dioxide elimination, like the oxygen consumption, rose promptly after the ingestion of coffee, tea and caffeine alkaloid, reaching a peak within 15 or 30 minutes after ingestion. The differences in the individual periods (fig. 1) were of no statistical significance; likewise the total increase above the basal for the entire experiment shown on the right of the curves was not significantly different in the three groups of experiments on either of the subjects. As in the case of oxygen consumption the increase in carbon dioxide elimination after taking coffee must be attributed to the caffeine content. The ingestion of decaffeinated coffee produced approximately the same effect as that previously observed after taking water, whereas when caffeine was added to decaffeinated coffee the effect was the same as that obtained with coffee.

Respiratory quotient

The curves of the respiratory quotients of the two subjects, shown in figure 1, present the same general features throughout the experiment. Ingestion of coffee, tea and caffeine alkaloid was followed by a prompt rise in the quotient of both subjects during the first 15-minute period, which continued and reached its peak in the second period, except in the experiments with tea on W. W. In these the peak of the rise was attained in the first 15-minute period. The quotient of C. E. rose more

abruptly in the first period and reached a greater height in the second period than in the case of W. W. Similar differences in the respiratory quotient of these two subjects after the ingestion of caffeine had been observed in earlier experiments ('41).

While there were some differences in the rise of the quotient above the basal in the various 15-minute periods after taking coffee, tea and alkaloid, the total effect on the quotient for the entire duration of the experiment was the same for the different groups of experiments. The respiratory quotient for 105 minutes after ingestion (seven 15-minute periods) in each group was 0.03 above the basal in one subject and 0.02 above in the other.

In the control experiments with decaffeinated coffee the quotients were practically the same as in numerous other experiments, not included in the present study, in which water alone was taken. The rise in the quotient of C. E. toward the latter part of the control experiments with decaffeinated coffee (which was the usual occurrence also in the experiments in which water alone was ingested) was probably due to a slight degree of restlessness because of the discomfort of lying still and breathing continuously through the mouthpiece.

Interpretation of the respiratory quotient

The effect of coffee and of tea on the respiratory quotient must be attributed solely to their caffeine content. This becomes obvious upon comparing the action of caffeine alkaloid with that of decaffeinated coffee with and without the addition of caffeine.

The interpretation offered for the behaviour of the quotient after the ingestion of caffeine in a previous study ('41) applies to the present experiments with coffee and tea. Carbohydrate oxidation was not increased, as is evident from the slight extent of the rise in the quotient above the basal for the entire experiment. This small rise of 0.03 and 0.02 was no greater than that which occurred in the control experiments with decaffeinated coffee and in previous experiments in which water alone was taken. For the same reasons adduced in our previous study on caffeine, it may be concluded that the marked rise in the quotient after taking coffee and tea was due to the hyperventilation caused by the caffeine content. The hyperventilation was a consequence of an increase in respiration and was completely compensated by retention of carbon dioxide before the conclusion of the experiment. (Cf. Haldi, Bachmann, Ensor and Wynn, '41).

Respiratory ventilation

The effects of coffee and tea on respiratory ventilation as shown in table 1, were the same as those produced by caffeine in solution. The marked rise in the minute volume of inspired air following the ingestion of the caffeine solution was similar to that obtained with the alkaloid in previous experiments ('41). There was a small increase in the experiments with decaffeinated coffee but this was practically the same as that which we have observed after the ingestion of the same amount of water.

TABLE 1

Respiratory ventilation (liters per minute at 0°C. and 760 mm. Hg.) as affected by caffeine taken in the form of alkaloid, coffee, and tea.

	BASAL	CONSECUTIVE 15-MINUTE PERIODS							AVG. INCREASE PER MIN.
		1	2	3	4	5	6	7	
Subject — W. W.									
Caffeine alkaloid	4.80	+1.02	+1.10	+ .90	+.81	+.72	+.60	+.77	.85
Coffee	4.84	+1.06	+1.13	+1.02	+.90	+.72	+.83	+.88	.93
Tea	4.90	+1.29	+ .94	+ .86	+.79	+.98	+.86	+.98	.96
Decaffeinated coffee	4.52	+ .52	+ .23	+ .09	−.05	+.14	+.23	+.41	.22
Decaffeinated coffee and caffeine	4.46	+1.01	+1.26	+1.00	+.88	+.84	+.71	+.80	.93
Subject — C. E.									
Caffeine alkaloid	5.30	+ .73	+1.24	+ .92	+.81	+.63	+.56	+.52	.77
Coffee	5.00	+ .86	+1.18	+ .84	+.69	+.59	+.64	+.68	.78
Tea	5.22	+1.13	+ .77	+ .93	+.91	+.87	+.87	+.72	.89
Decaffeinated coffee	5.10	+ .38	+ .09	+ .05	+.08	+.24	+.25	+.33	.20
Decaffeinated coffee and caffeine	5.23	+ .99	+1.30	+ .83	+.74	+.54	+.46	+.60	.78

It may therefore be concluded that the stimulating action of coffee and tea on respiratory ventilation was due solely to the caffeine content of these beverages.

SUMMARY AND CONCLUSIONS

A comparative study has been made of the gaseous exchange of two well trained subjects following the ingestion of 200 mg. caffeine alkaloid and of coffee and tea infusions containing this amount of caffeine.

The action of caffeine on oxygen consumption, carbon dioxide elimination, the respiratory quotient and respiratory ventilation was the same as that observed in previous experiments of this kind. Coffee and tea had the same effect as the alkaloid.

Comparison of these results with those obtained with decaffeinated coffee and decaffeinated coffee to which caffeine was added shows that the effect of coffee and tea was due solely to their caffeine content.

It may be concluded from these experiments that the rate of absorption and the action of caffeine in coffee and tea is the same as that of the pure alkaloid in solution.

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SOME OBSERVATIONS OF DARK ADAPTATION IN MAN AND THEIR BEARING ON THE PROBLEM OF HUMAN REQUIREMENT FOR VITAMIN A [1]

E. L. BATCHELDER [2] AND JANE C. EBBS [3]

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ONE FIGURE

(Received for publication November 22, 1943)

INTRODUCTION

In a previous paper, Batchelder and Ebbs ('42) reported the results of a study on the dark adaptation of college students and its relation to vitamin A metabolism. Some preliminary studies of vitamin A requirement were included which indicated that in three young women 5500 I. U. vitamin A was slightly more than their minimum requirement. The purpose of the work described below was to throw more light on the question of minimal needs for vitamin A. The few figures on requirements now found in the literature show for the most part the amount of vitamin A (or plant precursors) required to restore to normal an impaired visual threshold. Under such conditions some of the vitamin A might actually be used in repair during recovery and in storage, after the lowest threshold attainable by the individual has been reached. Such dietary requirement figures for vitamin A might therefore be expected to be somewhat higher than the minimal because storage could conceivably account for a fraction of the vitamin A intake. The more rapid the rate at which the visual threshold approaches the individual's optimum, the more likely it is that the vitamin A intake is exceeding the minimal requirement for maintaining that threshold. It seemed desirable therefore to approach the question of minimal dietary requirements from another standpoint in order to eliminate storage as a factor. The maintenance of visual thresholds somewhat above those shown by the subjects before changing to a vitamin A deficient diet has been studied for that purpose. The results are

[1] Contribution no. 645 of the Rhode Island Agricultural Experiment Station.

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here reported along with additional information on (1) time required on a depletion diet for onset of impaired visual sensitivity, (2) rate of rise in the visual threshold when depleted subjects were continued on a diet practically devoid of vitamin A value, (3) changes in the character of the dark adaptation curve as visual sensitivity decreased, and (4) return of depleted subjects to normal.

The results of the present experiment are not necessarily to be interpreted as indicating a practical dietary requirement (minimal vitamin A needed to support maximal visual sensitivity). The maintenance figures present values lower than the amounts which could apparently be used to advantage by the individuals studied.

EXPERIMENTAL

Technique

The instrument used for the measurement of dark adaptation was the Rhodometer (Batchelder and Ebbs, '42). The standard procedure previously used in survey tests establishing a normal range for several hundred college students was followed using both the 30- and the 15-minute thresholds as criteria of ability to adapt to darkness. The procedure involved light adaptation of 3 minutes immediately preceding the measurement of rate of dark adaptation. The minimum brightness perceived at different times during the dark adaptation was recorded and plotted as the logarithm of brightness in micromicrolamberts. An artificial pupil was used at all times.

Procedure

Three female subjects, M. W., E. G., and Y. H., and one male, R. Y., volunteered to go on a vitamin A-low diet. All underwent physical and dental examinations which showed them to be healthy young adults.

The subjects were placed on a diet calculated to yield below 200 I. U. of vitamin A per day. The selection of the foods low or lacking in vitamin A was made through the use of the tables of Daniel and Munsell ('37), Munsell ('40), and Booher and Marsh ('41).

The dark adaptation of each subject was measured at least three times weekly and practically daily during the time vitamin A supplements were fed.

The four subjects ate meals prepared in the laboratory kitchen and were served approximately uniform portions with the exception of vitamin A-free breads and sweets which were taken at will. All received 50 mg. of crystalline ascorbic acid plus 4 oz. of canned grapefruit

juice daily. Activated ergosterol was administered weekly in doses of 3,000 to 4,000 I. U. In addition, each subject received 6 brewers' yeast tablets daily. One pint of skimmed milk (0.02% butterfat) was consumed daily by each subject. The nature of the ergosterol capsules was unknown to the subjects who suspected vitamin A. Moreover, when vitamin A feeding was begun, all subjects received either placebos or supplements.

RESULTS

Time elapsing before signs of poor dark adaptation appear

Chart 1 shows the 30-minute threshold for each subject. A trend toward higher visual thresholds was observed in these subjects although in subject Y. H., an abrupt drop occurred on the eighty-fifth day and a low threshold was maintained from then to the end of the experiment. Subjects R. Y., M. W., and E. G. showed definite rise in visual threshold within the range of 16 to 124 days on the depletion diet as reported by Booher et al. ('39). The record of Y. H. is somewhat similar to that reported by Steffens et al. ('39), and Brenner and Roberts ('43), who reported that only slight and temporary rises in threshold were observed in subjects on a vitamin A-deficient diet for 6 months. None of our subjects showed the immediate rise in threshold following a vitamin A-free regime reported by Hecht and Mandelbaum ('40). Blanchard et al., ('40) reported that 10 to 31 days were necessary, and Batchelder and Ebbs ('42) found that 29 to 73 days on the diet were required before the threshold rose.

Rate of depletion and response to vitamin A supplements

All four subjects showed a sensitivity well within the normal range (Batchelder and Ebbs, '42) at the beginning of the experimental period. The rate of change in visual sensitivity varied among the four subjects.

Subject E. G. showed a markedly higher threshold on the eighty-first day, but otherwise showed only a slight, steady rise amounting to about 0.5-log unit by the one hundred and eighteenth day. This was not arrested by the feeding of 2,000 I. U. of vitamin A concentrate daily from the one hundred and eighteenth to the one hundred and forty-fifth day. The feeding of 4,000 I. U. daily (84 I. U. per kilogram of body weight) resulted in a drop in the threshold to a level similar to that which was shown at the beginning of the 2,000 I. U. feeding period. This level was 0.7- to 0.8-log unit above her threshold at the beginning of the experiment, and was maintained with few exceptions from the one hundred and forty-fifth to the one hundred and eightieth day on an intake of 4,000 I. U. of vitamin A daily.

Subject M. W. showed a marked rise in threshold between the seventy-fourth and seventy-sixth day, at which point it remained constant for about 10 days and then increased on the eighty-ninth day to approximately 1.0-log unit above her original threshold. The feeding of 2,000

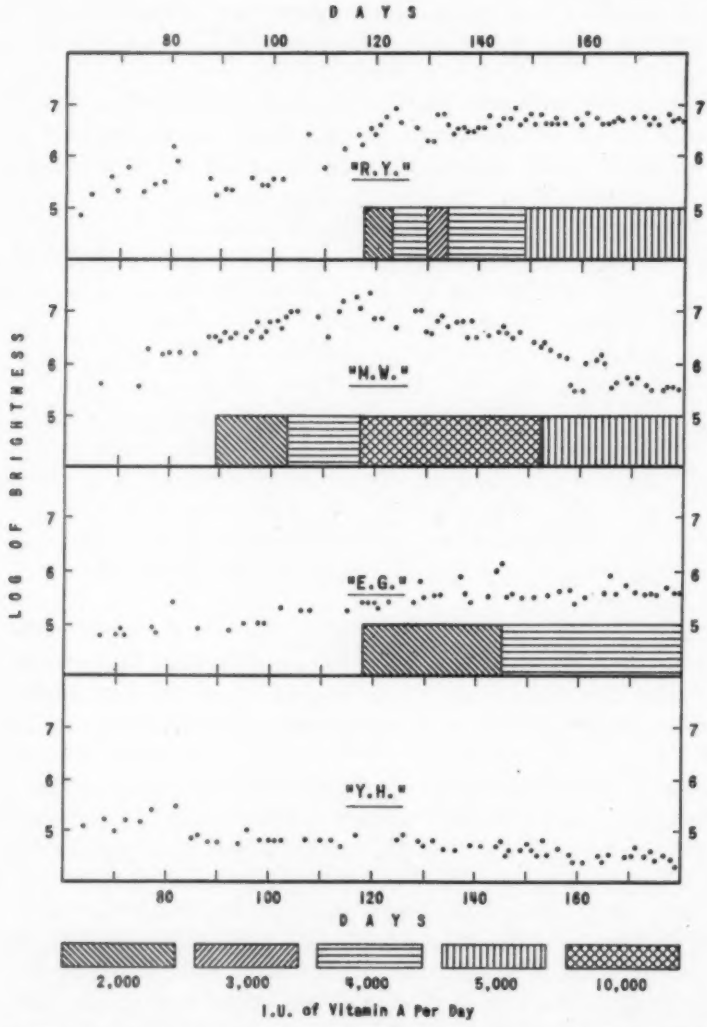

Fig. 1 Thirty-minute thresholds for perception of brightness at different times following a change from a freely chosen to a vitamin A-deficient diet and after feeding known amounts of vitamin A.

I. U. daily failed to check this rise. Blood vitamin A and blood carotene were within the normal range, 620 and 440 I. U. per 100 ml., respectively, on the ninety-ninth and one hundred and fourth day. In spite of the apparently normal blood picture, it was decided to increase the vitamin A supplement to 4,000 I. U. daily. The threshold dropped during the next 6 days but by the seventeenth day was higher than before (1.7-log units above the original level). On the one hundred and eighteenth day, the subject was emotionally unstable, and although she was unaware of the results of the tests, reported that she felt "helpless" and "all gone."

The vitamin A supplement was increased to 10,000 I. U. until the threshold reached the level at which the first feeding tests of the vitamin A concentrate were started. This level was reached on the one hundred and fifty-second day, at which time the feeding of 5,000 I. U. daily was begun. The threshold continued to drop for several days, probably due to residual stores from the previous period. From the one hundred and sixty-fifth day to the one hundred and eightieth day, the threshold was close to the subject's original dark-adaptation level. If the effect of previous higher feeding can be ruled out, this represented maintenance of a normal dark-adaptation threshold on an intake of 81 I. U. per kilogram of body weight. Return to a freely-chosen diet plus 10,000 I. U. of vitamin A daily for 45 days caused no further improvement in her threshold.

Subject R. Y. showed a rise in threshold from the sixty-fifth to the eighty-first day which was followed by a spontaneous drop which reached its lowest point on the eighty-ninth day. This was similar to the drop observed at about the same time in subject Y. H. and also less definitely in the record of E. G. A fairly steady increase in threshold followed in the case of R. Y. until, on the one hundred and seventeenth day, it was 1.5-log units above the normal level. A supplement of 2,000 I. U. daily for 6 days failed to check this increase, and 4,000 I. U. were fed. This resulted in a marked drop in the threshold in 7 days, at which point 3,000 I. U. were tried for 4 days. The threshold rose again, however, and the 4,000 I. U. level was resumed. A slow increase was evident after the one hundred and first day, and on the one hundred and forty-ninth day, the supplement was raised to 5,000 I. U. At this level of intake of vitamin A the dark-adaptation ability remained very constant to the one hundred and eightieth day. This, however, was a level almost 2.0-log units above the subject's normal dark adaptation level. At that date, the controlled vitamin A-deficient diet had to be discontinued and it was not possible to determine how

much was necessary in order to maintain his dark adaptation at a more favorable level. The 5,000 I. U. intake level represented 74 I. U. per kilogram and is inadequate for the maintenance of maximal dark adaptation for this subject. After the one hundred and eightieth day, he returned to a freely-chosen diet plus approximately 10,000 I. U. of vitamin A daily. Recovery was slow, however, and after 42 days he was given 300,000 I. U. daily. This resulted in a sharp drop. Subsequent feedings of this amount for 9 more days resulted in only slow recovery. The initial sharp drop following the first massive dose appears in line with our own previously reported experience and with that of several others cited by Rajogopal ('41).

Character of the dark-adaptation curve

During the early stages of depletion (when 30-minute dark adaptation thresholds were rising) the 15-minute thresholds tended also to rise, but the earlier thresholds, where cone vision was apparently functioning, did not rise until later in the depletion period.

DISCUSSION

The difficulties of presenting the results of dark adaptation tests in the form of tables have made it possible to present in detail only the 30-minute thresholds of the subjects during the part of the experimental period beginning just before the first significant change in dark-adaptation ability was observed. Chart 1 presents these data. It will be observed that after a period of about 2 months, during which the effect of the vitamin A-deficient diet was apparently compensated for by bodily stores of vitamin A, a rise in the thresholds occurred. The initial rise, which is interpreted as indicating that bodily stores have been depleted, was followed by a decided drop in three of the four subjects. This drop, in the case of E. G. and R. Y. was followed by a second gradual increase. The drop might be interpreted as a temporary adjustment of the body to a lower level of vitamin A metabolism, which could not be maintained in the face of a persistent shortage. In the case of Y. H., however, a second increase did not follow. The fourth subject, M. W., showed no temporary arrest in the trend toward poorer dark adaptation. Her response to the different levels of feeding chosen was very consistent. The fact that her dark-adapting ability appeared to improve for a considerable period after being changed from a 10,000 to a 5,000 I. U. supplement is interpreted as indicating that some storage of vitamin A had been possible.

The variation in the rate of depletion of different subjects may be partly due to individual differences in physiologic reponse to vitamin A deficiency, as in the case of one of our subjects and a few others reported in the literature who have failed to show a decrease in dark adaptation after many months on a vitamin A-deficient diet. Unusually large body stores of the vitamin, or more economical use of them are probably the chief reason for lack of evidence of deficiency after many months on a deficient diet.

SUMMARY AND CONCLUSIONS

Four young adults were fed on a diet deficient in vitamin A but adequate in all other known nutrients. Their dark adaptation was measured by means of the Rhodometer, previously described. One subject showed slight initial rise in visual threshold, but after the eighty-fifth day, showed a gradual improvement in dark adaptation up to the one hundred and eightieth day when the study was discontinued. The others showed a rise in visual threshold after 65 to 100 days. Certain aspects of the rate of change in visual threshold have been brought out. When the rise amounted to about 0.5-log unit, one subject was fed 2,000 I. U. of vitamin A daily for 27 days. Since the threshold continued to rise, the dose was increased to 4,000 I. U. This resulted in maintenance of a constant 30-minute threshold for about 45 days. Two subjects were treated in a similar manner except that the visual threshold was allowed to rise 1.0-log unit before vitamin A was fed. In both instances, 4,000 I. U. were inadequate for maintenance, but 5,000 I. U. served to maintain constant but subnormal dark adaptation for about 1 month. Individual differences in response to large doses of vitamin A and in return to normal visual sensitivity were observed.

In three different subjects, 74 I. U. vitamin A per kilogram body weight (5,000 I. U. per day) sufficed only to maintain a threshold 1.5-log units above normal; 84 I. U. per kilogram (4,000 per day) maintained a threshold 0.5-log units above normal; and 81 I. U. per kilogram (5,000 I. U. per day) maintained an approximately normal threshold.

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A STUDY OF NEUROMUSCULAR REGENERATION UNDER DIFFERENT LEVELS OF VITAMIN C INTAKES[1]

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Numerous reports have appeared which indicate the importance of an adequate intake of vitamin C for the proper healing of surgical wounds in man and the guinea pig. Information is relatively meager concerning the role of vitamin C in the regeneration of muscle and nerve tissues. Dalldorf ('29) reported that severe scurvy in experimental animals was accompanied by extensive muscle damage. Hojer ('24) considered such muscle lesions to be characteristic of scurvy. Kodicek and Murray ('43) noted that guinea pigs recovering from injury to bone and muscle on diets partially deficient in vitamin C exhibited muscle swelling and overgrowth of connective tissue. These investigators recommended a liberal supply of vitamin C to patients recovering from injury to bones and muscles.

The present investigation was designed to study the relation of vitamin C intake to muscle and nerve regeneration. Information was sought concerning the effects of suboptimal intakes and excess intakes of ascorbic acid upon the extent and velocity of muscle and nerve regeneration following peripheral nerve injury.

EXPERIMENTAL

A total of fifty young guinea pigs were selected from a common stock, matched as to age, body weight and sex and equally divided among five groups. The basal ration (Eddy, '29) used throughout these studies had the following percentage composition: baked skim milk powder, 30; butterfat, 9; sodium chloride, 1; rolled oats and bran (equal parts by volume), 59; and cod liver oil, 1. The above diet was supplemented with 1.5 mg. of alpha-tocopherol[2] per animal per day and with ascorbic acid in amounts of either 0.5, 1.0, 2.5, 5.0 or 50.0 mg.

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[2] The alpha-tocopherol was supplied through the courtesy of Hoffman-LaRoche, Inc., Nutely, N. J., and Merck and Company, Rahway, N. J.

per day. The vitamin supplements were administered orally from a pipette. A few days were allowed for the animals to become accustomed to their diets before operation.

A complete denervation of one gastrocnemius muscle was accomplished by exposing the tibial nerve under ether anesthesia and crushing it with a heavy linen ligature against a brass rod. This procedure allowed for complete denervation and preserved a good alignment for subsequent nerve regeneration. The unoperated contralateral muscle and nerve served as controls. Studies were made upon the muscles and nerves of the operated and control limbs at 35 days after denervation. At this time the regenerating nerves had established functional contacts with their muscles and the latter were recovering mass and strength which had been lost prior to reinnervation.

Measurements were made concerning the strength of the regenerating and contralateral control muscles. This was done by measuring the maximal isometric tension response to volleys of slightly supermaximal stimuli applied directly to the muscle and to its motor nerve. These operations were carried out under light ether anesthesia. The techniques employed for stimulation and strength measurements have been described in detail elsewhere (Hines, Thomson and Lazere, '42). It is to be noted that under these conditions the tension responses of control non-denervated muscles to nerve stimulation were of the same magnitude as those elicited by direct muscle activation. However, in the case of muscles undergoing reinnervation and regeneration, the tension responses of the muscles to nerve stimulation were only a fraction of those developed in response to direct activation. These techniques made it possible to determine quantitatively the extent of functional motor reinnervation as well as the total strength of the muscles of guinea pigs subsisting on different levels of vitamin C intakes. At the conclusion of the tension measurements, the gastrocnemii were carefully dissected and weighed in order to determine the degree of atrophy present at that time.

The experiments described above were not carried out on animals subsisting on less than 0.5 mg. of ascorbic acid per day, inasmuch as their survival time would have been too short and uncertain to permit comparable times for studies upon regeneration. In other experiments determinations were made of the strength, water, chloride and creatine concentration of gastrocnemius muscles from guinea pigs subsisting for 20 days on the basal C-deficient diet without supplements and from control animals whose diet was supplemented with 10 mg. of ascorbic acid per day.

EXPERIMENTAL RESULTS

A summary of the average values obtained from a study of forty-three animals appears in table 1. It is to be noted that non-denervated gastrocnemii from animals subsisting on different intakes of ascorbic acid, ranging from 0.5 to 50.0 mg. per day, exhibited essentially the same tension per unit of muscle weight. This finding was true for the responses to nerve stimulation as well as to direct muscle activation. The muscles of the control limbs appeared normal grossly and capillary hemorrhages were not observed. However, the non-denervated gastrocnemius muscles of scorbutic animals which had subsisted on a

TABLE 1

A summary of average values together with standard errors for the regenerating and control gastrocnemius muscles and tibial nerves of guinea pigs on different levels of ascorbic acid intakes. The measurements were made 35 days after a unilateral crushing of the tibial nerve.

NO. OF ANIMALS	DAILY INTAKE OF ASCORBIC ACID	TENSION PER GRAM MUSCLE WHEN ACTIVATED THROUGH				RELATIVE[1] STRENGTH OF REGENERATING MUSCLE WHEN ACTIVATED THROUGH		RELATIVE[1] WEIGHT OF REGENERATING MUSCLE
		Nerve		Muscle				
		Exper.	Control	Exper.	Control	Nerve	Muscle	
	mg.	*gm.*	*gm.*	*gm.*	*gm.*	%	%	%
9	0.5	368±37	1523±113	477±72	1533±174	18.9±4.9	29.8±7.4	85.8±6.9
10	1.0	515±57	1651±47	799±51	1652±56	28.2±2.6	44.7±3.6	91.3±4.1
8	2.5	780±39	1371±77	1191±30	1540±72	51.4±3.9	66.8±3.6	81.9±1.6
7	5.0	890±44	1581±100	1198±75	1660±94	45.2±4.9	57.9±5.7	82.1±2.4
9	50.0	945±53	1562±69	1117±39	1642±69	50.5±2.0	56.9±1.5	83.6±2.0

[1] Expressed as percent of values for non-denervated contralateral control.

C-deficient diet for 3 weeks were found to be weaker than comparable muscles of animals which had received adequate intakes of this vitamin (table 2).

Essentially the same degree of atrophy and strength per unit of weight was found for the regenerating gastrocnemius muscles of guinea pigs subsisting on a daily intake of 2.5 mg. as in the experiments with intakes as high as 50.0 mg. of ascorbic acid per day. The ratios of the tensions developed by the experimental muscles to that by their non-denervated controls were not affected by these different levels of vitamin C intakes. This was true for both the tensions elicited by nerve stimulation and by direct muscle activation. However, the tensions per unit weight for the regenerating muscles of animals receiving either 0.5 or 1.0 mg. of vitamin were consistently lower than those receiving the larger intakes. Likewise, the ratios of the tensions

developed by the regenerating muscles to those by their contralateral controls were lower than those observed for animals subsisting on ascorbic acid intakes usually regarded as optimal or excessive. The findings of lowered ratios for tension development for both nerve and direct stimulation indicate that subnormal intakes of ascorbic acid lead to impaired functional regeneration of muscle and nerve. The data (table 1) indicate that the extent of atrophy, when calculated on a wet weight basis, was less on the low ascorbic acid intake than on the higher levels of intake. Calculations of atrophy from differences in wet muscle weights may be misleading because the regenerating muscles of the animals on the lower ascorbic acid intakes exhibited numerous capillary hemorrhages which were not observed in their non-denervated contralateral controls. Hemorrhagic lesions were absent from both the control and regenerating muscles of animals receiving optimal or above

TABLE 2

A summary of average values for the gastrocnemii of guinea pigs on vitamin C-free diets and control diets with adequate intake of C.

DIET	NO. OF ANIMALS	TENSION PER GM. MUSCLE	CREATINE PER 100 GM. DRY WEIGHT MUSCLE	MUSCLE WATER	CHLORIDE PER 100 GM. MUSCLE
		gm.	*mg.*	%	*millimols*
Control	6	1703	2032	76.90	49.6
C-free	6	1404	2049	77.74	83.3

optimal intakes of ascorbic acid. An increased chloride and water concentration was observed (table 2) in muscles from animals subsisting on a scorbutogenic diet, the magnitude of which appeared to be correlated with the extent of capillary hemorrhage. When due allowance is made for weight changes due to such causes, it is apparent that the rate of regeneration of muscle mass is but little affected by the levels of ascorbic acid intakes. The creatine concentration per unit of dry muscle weight was precisely the same in the scorbutic animals which had subsisted on a vitamin C-free diet for 3 weeks as in the muscles of control animals on the same basic diet supplemented with 10 mg. of ascorbic acid.

DISCUSSION

The gastrocnemius muscles of guinea pigs lose about one-third of their mass and three-fourths of their original strength during the first 2 weeks following denervation. Soon thereafter the first signs of reinnervation appear, atrophy is checked and the affected muscles gradually recover mass and strength. Our findings indicate that the

regenerating muscles of guinea pigs subsisting on ascorbic acid intakes below those considered to be adequate for normal growth rates and protection against scurvy were functionally inferior to the regenerating muscles of animals receiving adequate intakes of the vitamin. This relative weakness of the regenerating muscles was observed for the response to direct as well as to nerve stimulation. In many instances these regenerating muscles exhibited numerous capillary hemorrhages and stood in contrast to the normal appearance of their contralateral controls and to the regenerating muscles of animals supplied with adequate amounts of vitamin C.

Only speculation can be advanced for the presence of hemorrhagic lesions and retarded functional recovery in the regenerating muscles of animals on suboptimal intakes of vitamin C. The crushing of the peripheral nerves deprived the muscles of their sensory as well as their motor innervations and caused a loss of protective reflexes. This resulted in the affected muscles being more vulnerable to stress, strain and trauma. A lack of formation of the proper quality or quantity of collagenous intercellular substances in the supporting tissues may well have contributed to the retarded functional recovery of muscle and nerve from the combined effects of denervation and trauma. The ratios of the tensions developed in response to stimulation of the regenerating nerves to those from direct muscle activation were not significantly different in the animals subsisting on different levels of ascorbic acid intakes. This finding suggests that the relative weakness of the muscles of animals subsisting on the lower levels of vitamin C was not due to ineffective reinnervation but rather to conditions inherent in the muscles themselves. In view of the fact that the lesions of scurvy are much slower to appear in fully grown animals than in growing animals (Wolbach and Bessey, '42), it is possible that the minimal vitamin C requirements are higher for regenerating muscle than for non-regenerating tissue.

Intakes of ascorbic acid considerably in excess of those recognized to be adequate for normal growth and protection against scurvy had no beneficial effect upon the course of neuromuscular regeneration. Our findings indicate that the optimum intake of vitamin C for promoting recovery from peripheral nerve injury is not appreciably different from that recognized to be adequate for the organism as a whole.

SUMMARY

A comparative study was made of neuromuscular regeneration in guinea pigs subsisting on different levels of vitamin C. The ascorbic

acid intakes ranged from 0.5 to 50.0 mg. per day. Complete denervation of the gastrocnemius muscle was produced by crushing the tibial nerve. The contralateral non-denervated limb served as a control. Studies were made at 35 days after operation concerning muscle atrophy and strength and the capacity of the regenerating nerve to activate its muscle.

The regenerating muscles of animals subsisting on suboptimal intakes of vitamin C were relatively weaker than those of animals supplied with adequate intakes. It is suggested that the retarded functional recovery in such muscles may be due to a lack of sufficient collagenous material for regeneration and protection against hemorrhagic lesions precipitated by stress, strain and trauma. The possibility also exists that regenerating muscles may have a higher vitamin C requirement than non-regenerating control muscles. It was found that excess intakes of vitamin C had no beneficial effect upon the course of neuromuscular regeneration.

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RESPONSE TO THE INTRAVENOUS INJECTION OF ASCORBIC ACID AS INDICATED BY THE URINARY EXCRETION OF THE TOTAL AND REDUCED FORMS

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The response to a load or saturation test for ascorbic acid is usually reported in terms of the reduced form of the vitamin. Actually, however, there is no evidence of which the authors are aware, to indicate that the quantitative excretion of the dehydro form of the vitamin might be so negligible as to be unimportant. Although our preliminary work showed that in fasting basal urine samples a rather constant amount (10–20%) of the total excretion of ascorbic acid existed in the dehydro form, there remained the possibility that the same relative results might not obtain following the intravenous injection of 200 mg. of ascorbic acid. The proper interpretation of the response to this load test depends upon a knowledge of the total excretion of ascorbic acid, i. e., reduced plus dehydro forms.

The recent publication of Roe and Kuether ('43) describes a method for the estimation of total ascorbic acid. When this is used in conjunction with the determination of reduced ascorbic acid, the dehydro form can be estimated by difference. Consequently, the matter of the relationships between total, reduced and dehydro ascorbic acid excretion following a load test has been investigated as part of a study of the nutritional status of soldiers, and the results are the subject of this report.

METHODS

The subjects were sixty-eight officers and enlisted men ranging in age from 18 to 42 years, in weight from 128 to 236 pounds, and in height from 5 feet 5 inches to 6 feet 2 inches. For 10 days immediately preceding the load test, all personnel had subsisted on Army Field Ration B which furnished an average daily intake of approximately 100 mg. of ascorbic acid (as determined from actual food consumption and chemical analyses of the food). During the 3 weeks preceding that

time, all had subsisted solely on Army Field Ration A (i. e., the rough equivalent of a liberal civilian diet) in one mess hall. During this time the calculated average daily ascorbic acid intake was in excess of 100 mg.

The following load test procedure was adopted to avoid any interference with the routine military activities of the subjects as well as to avoid error attendant on inclusion of the post prandial excretion of the vitamin. The evening meal, from which all foods high in ascorbic acid had been removed, was eaten at about 6 P. M. At midnight the men were awakened, they emptied their bladders completely, and received intravenously 200 mg. of ascorbic acid,[1] which was administered by the attending medical officer. The intravenous route of administration was selected as preferable to the oral in that it obviated variability in intestinal absorption or destruction. At 6 A. M., the men were again awakened, and excreted the 6-hour urine sample into vessels containing a weighed amount of solid metaphosphoric acid sufficient to prepare a final 2% solution upon further dilution of the urine to a convenient volume. The urine samples were at once transported to the laboratory for immediate analysis.

Reduced ascorbic acid was determined by the photoelectric, 2, 6-dichlorophenol indophenol method of Evelyn, Malloy and Rosen ('38), with a Coleman spectrophotometer. Total ascorbic acid was determined by the colorimetric method of Roe and Kuether ('43) with a Hellige photoelectric colorimeter. In our experience, concentrations of ascorbic acid less than 15 μg. per milliliter of urine did not produce enough color for accurate estimation by this technique. For this reason, excessive dilution of the urine was avoided, but an appreciable urine volume (200 ml. for the 6-hour period) was encouraged by the administration of a full glass of water at the start of the collection period, whenever the previous day had been hot. It was believed that urine volume of this size would, in addition, serve as a further precaution against possible errors in sampling and from failure to empty the bladder completely. The rare cases of gross deficiency in bladder emptying were detectable by the routine determination of creatinine, which was carried out by a photometric modification of the colorimetric methods of Folin and Wu ('19) and Peters ('42).

RESULTS AND DISCUSSION

A wide variation was noted among individuals in their response to the load test, despite a fairly equal and constant intake of ascorbic

[1] Injectable "Cenolate" Abbott ampules testing 104% of labelled strength were used; 4 ml. of the solution being taken up in a sterile syringe with 4 ml. of normal sterile saline.

acid. This was apparent in the estimation of both reduced and total ascorbic acid excretions (table 1). There is a very high correlation ($r = 0.914$) between the two methods, indicating a regular association of high reduced ascorbic acid excretion with high total ascorbic acid

TABLE 1

Excretion of total, reduced and dehydro ascorbic acid following intravenous injection of 200 milligrams of ascorbic acid.

SUBJECT NO.	TOTAL ASCORBIC ACID [1]	REDUCED ASCORBIC ACID [2]	DEHYDRO ASCORBIC ACID [3]	SUBJECT NO.	TOTAL ASCORBIC ACID [1]	REDUCED ASCORBIC ACID [2]	DEHYDRO ASCORBIC ACID [3]
	mg./6 hrs.	*mg./6 hrs.*	*mg./6 hrs.*		*mg./6 hrs.*	*mg./6 hrs.*	*mg./6 hrs.*
1	6.3	3.0	3.3	36	75.0	70.0	5.0
2	48.3	58.5	—10.2	37	8.5	7.6	0.9
3	75.0	83.7	— 8.7	38	30.0	29.2	0.8
4	37.6	33.4	4.2	39	13.8	12.7	1.1
5	71.3	19.9	51.4	40	45.0	43.0	2.0
6	12.0	10.6	1.4	41	8.5	9.4	— 0.9
7	20.0	17.0	3.0	42	31.5	29.8	1.7
8	6.0	4.2	1.8	43	4.3	10.2	— 5.9
9	7.5	5.1	2.4	44	6.0	6.0	0
10	38.8	35.4	3.4	45	20.0	23.0	— 3.0
11	47.5	43.0	4.5	46	69.3	61.5	7.8
12	6.8	5.1	1.7	47	8.3	8.7	— 0.4
13	49.8	48.5	1.3	48	14.8	13.4	1.4
15	9.2	6.8	2.4	49	4.5	7.2	— 2.7
16	9.0	7.6	1.4	50	21.5	18.3	3.2
17	17.8	17.0	0.8	51	7.0	7.0	0
18	10.2	5.2	5.0	52	18.0	14.0	4.0
19	6.4	3.4	3.0	56	4.9	12.7	— 7.8
20	5.6	0.7	4.9	57	46.5	43.3	3.2
21	5.5	4.7	0.8	58	66.8	61.5	5.3
22	6.5	4.3	2.2	59	8.5	11.3	— 2.8
23	4.5	4.7	— 0.2	60	46.3	40.2	6.1
24	7.3	7.6	— 0.3	61	66.5	63.0	3.5
25	5.4	7.2	— 1.8	62	9.0	5.2	3.8
26	32.0	22.8	9.2	63	19.0	13.0	6.0
27	7.5	3.9	3.6	65	42.0	7.9	34.1
28	99.6	63.1	36.5	71	77.6	24.0	53.6
29	80.7	63.6	17.1	72	60.0	53.0	7.0
30	31.5	23.8	7.7	73	60.9	48.6	12.3
31	64.1	59.2	4.9	74	95.0	63.6	31.4
32	6.5	5.2	1.3	75	8.0	0	8.0
33	30.5	27.8	2.7	76	12.5	2.6	9.9
34	8.5	9.8	— 1.3	77	5.3	0	5.3
35	6.3	9.8	— 3.5	78	103.5	102.0	1.5
				Means	29.4	24.2	5.2 [4]

[1] Method of Roe and Kuether ('43).
[2] Method of Evelyn, Malloy and Rosen ('38).
[3] By difference, total — reduced.
[4] Highly significant mean difference.

excretion. It can be concluded, therefore, that either method would prove equally reliable in determining response to the load test. If it should become apparent that no additional information is provided by the estimation of total ascorbic acid, the choice of method then becomes an individual matter. In our own experience, we have found the estimation of reduced ascorbic acid to be less time-consuming and more suitable for large numbers of urine analyses.

With few exceptions, the dehydro ascorbic acid (determined by difference), was small and rather constant from one man to another, representing, on the average, 18% of the total ascorbic acid excretion.

The difference between the means of the two methods, although small, is highly significant statistically as shown in the analysis of variance table (table 2). This is interpreted as indicating that a small

TABLE 2

Analysis of variance.

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE
Total	135	91,396	
Between men	67	86,093	1,285
Between methods	1	907	907
Error	67	4,397	66

but nevertheless significant quantity of dehydro ascorbic acid is actually present in addition to the reduced ascorbic acid measured in the total ascorbic acid method. An inspection of table 1 reveals six erratic dehydro ascorbic acid values, i. e., subjects 5, 28, 29, 65, 71 and 74, that might be suspected of being responsible for the significance of the difference between the means of the two methods. When these values are eliminated, however, the difference between the means, although reduced, is still highly significant statistically.

SUMMARY

Reduced and total ascorbic acid urinary excretions were determined on sixty-eight soldiers during a 6-hour period following intravenous injection of 200 mg. of ascorbic acid. Dehydro ascorbic acid has been computed by difference.

The excretion of total ascorbic acid paralleled the excretion of reduced ascorbic acid (correlation $r = 0.914$). It is concluded that the measurement of either substance would prove suitable to determine relative load test response.

The means of the total ascorbic acid and the reduced ascorbic acid excretion were 29.4 and 24.2 mg. respectively. The difference between the means of the two methods, although small, is highly significant statistically. This is interpreted as indicating the presence of a small quantity of dehydro ascorbic acid in the urine.

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A SURVEY OF THE ASCORBIC ACID STATUS OF COLLEGE STUDENTS

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ONE FIGURE

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Blood plasma ascorbic acid values of college students in four northwestern universities have been investigated by Brown and others ('43). A similar study was begun in 1941 at the University of Tennessee. The plan was to supplement and generalize the findings of the northwest group by sampling a population differing in locality and food habits.

EXPERIMENTAL

Subjects were college women enrolled in nutrition and physiological chemistry classes open to freshmen, juniors, and seniors. For the determination of the ascorbic acid content of the blood plasma the micromethod of Mindlin and Butler ('38) was used with the Bessey method ('38) of correcting for turbidity. The suitability of this method for survey studies has been verified by Beebe ('42). Blood samples were taken on two non-consecutive mornings after a breakfast containing no ascorbic acid, and all data reported are average plasma ascorbic acid values. A routine record was made of the previous day's dietary and the ascorbic acid content estimated.

RESULTS

A total of 345 students served as subjects of whom 196 were freshmen and 149 juniors and seniors who showed similar findings. The study extended from the spring of 1941 through the spring of 1943. Ascorbic acid values of the two groups were arranged according to the classification of Neuweiler ('39), (table 1). Its use does not imply agreement with Neuweiler's interpretation of the significance of these groupings but does simplify comparison of these findings with those of the northwestern survey which have been so classified.

The distribution of these data showed that approximately 60% of both groups lay within the middle two intervals. Almost 25% of the

freshmen and only 10% of the upper-classmen had plasma levels below 0.4 mg. %. The upper-class group had one-third of their number in the high level above 1.0 mg. %, the freshmen only one-sixth.

The means of the two groups, 0.67 and 0.84 mg. %, as well as the distribution of values indicated a difference in the two groups. Whether such differences of means could exist and still represent a homogeneous population was checked by Fisher's t test. The t value found, 4.85, exceeded the t value, 2.59, given for the 1% level for this size sampling. It may be concluded, therefore, that the two groups represented populations which were different with respect to their ascorbic acid status as shown by blood plasma values and that the upper-classmen had a

TABLE 1

Distribution of ascorbic acid plasma levels for Tennessee women compared with totals for women in the Northwest.

PLASMA ASCORBIC ACID	FRESHMEN		UPPER-CLASSMEN		NORTHWEST TOTALS FOR WOMEN	
mg./100 ml.	*No.*	*%*	*No.*	*%*	*No.*	*%*
Less than 0.4	47	24.0	14	9.4	36	7.6
0.4–0.79	82	41.8	56	37.6	199	42.3
0.8–0.99	33	16.8	29	19.5	124	26.3
Over 1.0	34	17.3	50	33.6	112	23.8
Mean plasma ascorbic acid, mg./100 ml.	0.67		0.84		0.797	
Standard error	± .024		± .026		± .0126	

significantly higher level. The mean values and the distribution of these data are similar to those found by the northwest workers, the freshmen tending to parallel their data for men, the upper-class group their data for women.

The results obtained by calculating unweighed food intakes are open to considerable doubt. Approximately 60% of these subjects ate at the university cafeteria and consequently standardization of servings could be assumed. The research students who recorded and calculated the dietaries were familiar with methods of preparation and were competent to weigh values in estimating vitamin content. No claim is made that the values given are more than fair estimates. Dietary intake was based on an average of the days immediately preceding the two blood samplings. There seemed to be little agreement between intake and the following day's plasma value. Averaging the two sam-

plings tended to improve agreement. The average intakes were close together, for freshmen 86.3 ± 3.14 mg. and for upper-classmen 82.9 ± 2.65 mg. There was no significant difference between these two intakes and from this portion of the data it appeared that the two groups had a different ascorbic acid status as shown by the plasma values on identical intakes.

It has been generally assumed that there is close correlation between dietary intake of ascorbic acid and plasma levels. In this study the correlation coefficient, r, was 0.376 for freshmen and 0.398 for upper-classmen. These indicate that the effect of intake in fixing plasma values was only 40%, if a straight line relationship is assumed. An

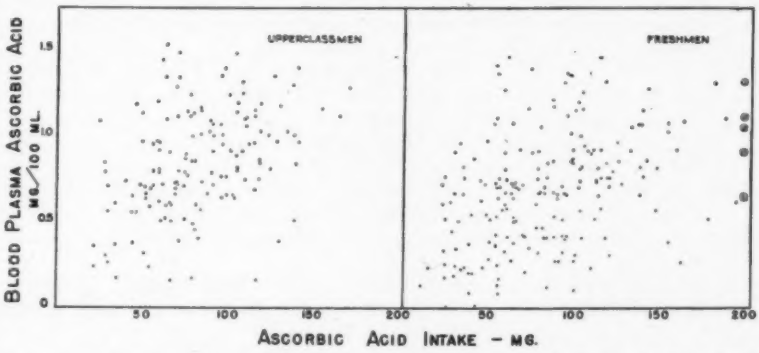

Fig. 1 Ascorbic acid intake and blood plasma values. Circle indicates intake of 200 mg. or above.

r value of 0.51 has been reported by Smith et al. ('42) for 300 subjects in a similar survey in which intake was calculated for 1 week before taking the blood sample. This longer period can be assumed to furnish a more representative estimate of ascorbic acid intake yet the correlation for the two factors was not greatly altered. A carefully controlled intake would be necessary to determine accurately the degree of this dependence, but the present data suggest the existence of other factors which affect a direct relationship of ascorbic acid intake to plasma values. Using Fisher's t test as a criterion, such correlation as exists is highly significant and cannot possibly be due to chance. Scatter diagrams are given for the two groups (fig. 1).

SUMMARY

1. Blood plasma ascorbic acid values obtained by a survey of 345 college women showed the freshmen to have a mean value of 0.67

mg. % which was significantly lower than the mean value of 0.84 mg. % of the upper-classmen.

2. The values obtained in this survey agree well both in means and distribution with findings of other workers using comparable methods of analysis for similar populations differing in locality.

3. Correlation based on a linear relationship of estimated dietary intake of ascorbic acid to blood plasma levels was limited and suggested that (a) such procedure in estimating dietaries is unsatisfactory and/or (b) other factors operate in this relationship.

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THE MAINTENANCE OF ADULT RATS ON DIETS LOW IN CERTAIN B VITAMINS[1]

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It appears to be generally accepted that a moderate excess of the essential dietary factors will promote better health and resistance to disease than a diet deficient in any one of them. However, Rasmussen ('43) has observed that mice deficient in thiamine were more resistant to the Lansing strain of poliomyelitis virus than those receiving adequate amounts of this vitamin. Rats on a diet deficient in the vitamin B complex are reported to be less susceptible to chronic ulcerative cecitis (Bloomfield and Lew, '43) or to infection by trypanosomes (Reiner and Paton, '32) than those on a more adequate diet.

Du Vigneaud and coworkers ('42) reported an increased incidence of hepatic tumors due to p-dimethylaminoazobenzene when biotin was added to the basal ration. Miner et al. ('43) observed that adult rats fed the dye and 25 μg. of pyridoxine per day developed more hepatic tumors than similar animals fed only 2 μg. of pyridoxine per day. Diets low in pyridoxine tended to diminish both the number of tumor "takes" and the growth rates of the inoculated tumors in rats and mice (Bischoff, Ingraham, and Rupp, '43; Kline et al., '43). Spontaneous mammary carcinomas in C_3H mice were found to grow more slowly when the host animal was fed a diet low in pantothenic acid (Morris and Lippincott, '41), in lysine (Voegtlin and Thompson, '36); Voegtlin and Maver, '36) or in cystine and methionine (Morris and Voegtlin, '40) than when adequate diets were fed.

Apparently, therefore, pathological conditions exist in which a mild, tolerable dietary deficiency may be preferable to the margins of safety usually prescribed. It is possible, for instance, that levels of nutrients may be found that are lower than the minimum needed for the growth of a virus or tumor, and yet are adequate for a reasonable degree of maintenance of the adult. However, information on the latter requirement is still too meager for an effective evaluation of the possibilities.

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To this end data have been assembled on the health and survival of adult rats fed synthetic diets low in each of the essential B vitamins.

METHOD

Groups of weanling albino rats of the Sprague Dawley strain were placed in screen cages and allowed to grow for 8 to 10 weeks on synthetic diets. They were then placed in single cages and fed "maintenance" diets deficient in individual vitamins of the B complex for a year or until death. Regardless of the condition of the animals this latter period was designated as the "maintenance" period. Food and water were given ad libitum, but the food consumption of each rat was measured bimonthly. The rats were weighed and examined weekly.

TABLE 1

Micrograms of the B vitamins fed per rat per day during the period of growth.

VITAMIN	DIET		
	Low vitamin	Medium vitamin	High vitamin
Nicotinic acid	10	30	60
Thiamine chloride	10	30	60
Riboflavin	15	45	90
Calcium pantothenate	44	132	264
Pyridoxine hydrochloride	10	30	60
Chlorine chloride	10000	10000	10000

The basal ration for both growth and maintenance contained purified casein,[2] 18; corn oil, 5; Wesson salts, 4; and glucose,[3] 73; plus various amounts of crystalline thiamine chloride, nicotinic acid, calcium pantothenate, riboflavin, pyridoxine hydrochloride, and choline chloride. These vitamins were mixed with glucose before being added to the other ingredients of the diet. The daily allotment of vitamins was contained in 6 gm. of the growth ration until the rats consumed 10 or more grams of food per day; thereafter, and during the maintenance period, this quantity of vitamins was added to 10 gm. of ration. The diets were mixed in 2- to 5-kg. lots and stored at $-5°C$. Each rat received 1 drop of halibut liver oil per week.

The diets fed during the growth period contained either low, medium, or high amounts of the B vitamins in a ratio of 1 to 3 to 6 times the lowest level (table 1). The medium vitamin diet was adopted as the

[2] Crude casein was washed for 1 week with several changes of tap water and then extracted with two changes of ethyl alcohol for 4 days at 50°C.

[3] Cerelose, a pure commercial glucose monohydrate.

control ration for the maintenance period, except that only 0.3 mg. of choline was contained in 10 gm. of ration instead of the 10 mg. fed during growth. The other maintenance diets differed from this ration in that one or more of the crystalline vitamins were omitted entirely. Such rations were designated as "free" from the factors in question. However, by the methods of analysis indicated each gram of casein contained 0.6 μg. of thiamine chloride (thiochrome), 0.6 μg. of riboflavin (fluorometric), < 1 μg. of pantothenic acid [4] (growth of Lactobacillus casei), and 0.2 μg. of pyridoxine hydrochloride (growth of Saccharomyces carlsbergensis). The glucose used was a highly purified product prepared by hydrolysis; it contained less than 1 mμg. of pyridoxine per gram as determined by the growth of yeast. Apparently, therefore, no rat received more than 1.1 μg. of thiamine, 1.1 μg. of riboflavin, 1.8 μg. of pantothenic acid, or 0.3 μg. of pyridoxine per day from the dietary constituents other than the crystalline vitamins.

EXPERIMENTAL

Growth period

During the growth period the rats on the medium and high vitamin diets gained 17 and 18 gm. per rat per week and reached average weights of 179 and 193 gm., respectively; those on the low vitamin diet gained an average of only 12 gm. per rat per week to a final weight of 143 gm. On a similar diet in which 8% of the glucose was replaced by brewers' yeast the rats weighed 242 gm. after 6 weeks (33 gm. per rat per week), although each of the crystalline B vitamins except nicotinic acid was present in lower concentrations in the yeast diet than in the synthetic vitamin diet (Sugiura and Rhoads, '41). The markedly greater rate of growth on the former diet, therefore, was probably due to other dietary essentials in the yeast.

Maintenance period

The changes in weight and survival of the rats on the various maintenance diets are indicated in table 2. The rats on the control diet continued to grow for the first 3 months of the maintenance period and thereafter maintained their weights. Those grown on the low vitamin diet tended to make up for their previous deficiency in vitamins and in size by gaining 133 gm. during the first 3 months of maintenance; those

[4] This determination was made by Dr. A. L. Neal. Under the conditions used 1 μg. of pantothenic acid per gram of casein was the smallest amount which could be determined.

grown on the medium level gained one-half as much and those raised on the high level one-fourth as much. In contrast to the animals on the various deficient diets (see below) most of the control animals remained in a good general state of health, although some developed respiratory trouble and died comparatively early in the experiment. After a year

TABLE 2

The maintenance of adult rats on deficient diets.

MAINTENANCE DIET	AVERAGE INITIAL WEIGHT	AVERAGE GAIN IN WEIGHT FROM INITIAL WEIGHT [1] AT			
		4 mo.	6 mo.	8 mo.	12 mo.
	gm.	gm.	gm.	gm.	gm.
Series I: Grown on the high vitamin diet.					
Control	189 (5)	35 (4)	40 (4)	40 (3)	31 (3)
Thiamine-free	170 (3)	.. (0)	..	..	..
Riboflavin-free	197 (3)	—22 (3)	—39 (3)	— 9 (2)	.. (0)
Pantothenic acid-free	200 (6)	12 (5)	18 (3)	.. (0)	....
Pyridoxine-free	200 (5)	26 (5)	14 (5)	16 (5)	— 1 (5)
Series II: Grown on the medium vitamin diet.					
Control	175 (6)	64 (6)	63 (3)	65 (3)	95 (3)
Nicotinic acid- and choline-free	182 (7)	58 (7)	50 (6)	90 (3)	131 (3)
Thiamine-free	193 (3)	.. (0)	..	..	..
Riboflavin-free	192 (3)	14 (3)	— 8 (3)	— 5 (3)	—68 (1)
Pantothenic acid-free	175 (8)	31 (7)	1 (3)	— 2 (3)	.. (0)
Pyridoxine-free	174 (8)	28 (6)	2 (5)	— 2 (2)	.. (0)
Series III: Grown on the low vitamin diet.					
Control	141 (3)	133 (3)	152 (3)	207 (2)	185 (2)
Nicotinic acid- and choline-free	134 (4)	108 (3)	118 (3)	127 (3)	92 (3)
Thiamine-free	164 (3)	.. (0)	..	..	..
Riboflavin-free	158 (2)	— 6 (2)	—14 (2)	.. (0)	..
Pantothenic acid-free	134 (5)	33 (5)	26 (2)	15 (1)	21 (1)
Pyridoxine-free	134 (5)	59 (5)	55 (4)	58 (3)	62 (1)

[1] Numbers in parentheses indicate the number of animals alive at each period.

of maintenance half of the males weighed 330 gm. and over, while half of the females exceeded 230 gm. in weight.

The omission of both nicotinic acid and choline from the maintenance ration failed to affect the performance of the adult rats. They continued to gain in weight slowly for about 3 months, and most of them still appeared healthy after a year. The deaths that occurred in this group were usually due to respiratory infections. Since the rat appears to be able to synthesize enough of both nicotinic acid (Dann, '41) and

choline (Jacobi, Baumann, and Meek, '41) for a fair rate of growth, it is not surprising that the synthesis of these factors should also be adequate for the maintenance of the adult. In fact for the purpose of the present experiment the rats maintained in the absence of dietary nicotinic acid and choline could be regarded as additional control animals.

In contrast the adult rats deprived of thiamine failed rapidly. Those grown on the diets of low or medium vitamin content gained in weight for only a week after being placed on the maintenance ration free of thiamine. Thereafter they declined rapidly to death in 2 to 4 weeks. When placed on the deficient ration, rats grown on the diet high in thiamine gained in weight for about 2 weeks and survived for a total of 6 to 7 weeks. During the last 2 or 3 weeks of life the rats consumed only 1 to 4 gm. of food per day; they became badly emaciated and many showed poor coordination.

The performance of the rats on the diets deficient in riboflavin, pantothenic acid, or pyridoxine was intermediate between that of the control animals and those deprived of thiamine. Significantly, many of the animals on each of these diets maintained their weights and remained free of gross deficiency symptoms for long periods of time. The rats on the diet free of riboflavin survived for 7 to 12 months. During the first 4 months these rats appeared nearly normal except for a slight loss in weight and lack of activity. Thereafter an alopecia gradually developed until, in some cases, most of the hair on the head, legs, and abdomen was gone. At the same time a dermatitis developed and became progressively more severe. Before death seven of the eight rats had open sores on the neck and paws and occasionally on other parts of the body.

The adult rats fed the diet free of pantothenic acid grew for about 1 month and thereafter either maintained their weights or declined very slowly. The animals remained approximately normal in appearance for 2 to 3 months, after which the chief gross symptoms were a listlessness and lack of muscle tone. "Bloody" noses, whiskers, and forelegs developed in about 70% of the animals before death. Survival was good up to 4 months, but about 50% of the animals died during the fifth and sixth months. Severe losses in weight were observed only during the last 10 to 14 days of life, and on autopsy numerous hemorrhagic areas were found in the intestinal tract, especially in the ileum. No macroscopic hemorrhages were found in the adrenals. Essentially the same results were obtained whether the rats had been grown on diets containing low, medium, or high amounts of the B vitamins.

This conclusion was also drawn from an experiment in which two other groups of weanling rats were grown on the diet free of pantothenic acid plus daily oral supplements of the vitamin in water. One group received 264 μg. of calcium pantothenate daily by dropper throughout the period of growth, the other 264 μg. daily during the first week with the subsequent doses decreased each week by 44 μg. per day. During the eighth week the daily dose was 22 μg. The rats on the graded dosage grew nearly as well as those fed the high level throughout (the weights after 8 weeks averaged 177 gm. and 206 gm., respectively), and when placed on the maintenance ration free of pantothenic acid, they survived 8 months as compared to 6 months for those receiving the larger doses of the vitamin. Unna and Richards ('42) observed that the requirement of the rat for pantothenic acid decreases with age.

By way of contrast, the maintenance of adult rats on the diet free of pyridoxine appeared to depend upon the amount of pyridoxine consumed during growth. Rats grown to maturity on the higher level of the B vitamins gained in weight during the first 2 months of maintenance and thereafter virtually maintained their weights for another 8 months on the deficient diet. They remained nearly normal in appearance for about 5 months when a loss in muscle tone became the most conspicuous symptom of deficiency. After 12 months on the deficient diet the survival of the animals was still 100%. However, of the rats grown on the low and medium diets 50% died within 9 and 7 months, respectively. In these two latter groups the rats gained in weight for about 1 month, lacked muscle tone after 3 to 4 months, and became hypersensitive in 5 to 6 months. One rat was observed in convulsions after 7 months.

Rats which had consumed only 0.2–0.3 μg. of pyridoxine daily for 2 months or more excreted 0.1–0.4 μg. of pyridoxine daily in the urine and 0.5–0.8 μg. in the feces, as determined by the growth of yeast (Atkin et al., '43). Control rats receiving 36 μg. of pyridoxine daily excreted about 30 μg. in the urine; after 1 day on the deficient diet, they excreted 3–8 μg. during the next 24 hours.

In another series twenty-six weanling rats were grown on the synthetic diet free of pyridoxine plus aqueous supplements of the vitamin orally. One group received 60 μg. of pyridoxine per day while two other groups received graded doses of the vitamin (table 3). The three groups gained an average of only 14, 10, and 9 gm. per week to average 168, 138, and 130 gm. in weight, respectively, at 9 weeks, as contrasted with an average weight of 193 gm. when 60 μg. of pyridoxine was incorporated into the daily allotment of the basal ration. The discrepancy

in growth rates was attributed to the rapid excretion of aqueous doses of pyridoxine (Scudi, Buhs, and Hood, '42, Scudi, Unna, and Antopol, '40; Swaminathan, '41).

After 9 weeks of growth the rats were placed either on the maintenance ration free of added pyridoxine, or on a diet containing 2 μg. of crystalline pyridoxine per 10 gm. of ration, or on the control ration containing 30 μg. of the vitamin per 10 gm. The maintenance of the rats on the diet deficient in pyridoxine again appeared to depend mainly upon the amount of this vitamin previously consumed. Only

TABLE 3

The effect of pyridoxine consumption during growth on the subsequent maintenance of rats deprived of pyridoxine.

GROWTH PERIOD		MAINTENANCE PERIOD		
Daily oral dose of pyridoxine	Average initial weight	Average gain in weight from initial weight [1] at		
		4 mo.	6 mo.	8 mo.
μg.	*gm.*	*gm.*	*gm.*	*gm.*
			No pyridoxine	
60 throughout	171 (4)	0 (4)	5 (4)	— 18 (3)
60, 50, 40, 30, 20 [2]	135 (2)	— 6 (2)	(0)	...
15, 12.5, 20 [2]	135 (5)	10 (2)	7 (2)	— 14 (1)
		2 μg. pyridoxine per 10 gm. maintenance diet		
60 throughout	166 (4)	22 (4)	31 (4)	22 (4)
		30 μg. pyridoxine per 10 gm. maintenance diet		
60 throughout	168 (4)	54 (4)	60 (4)	67 (4)
60, 50, 40, 30, 20 [2]	143 (2)	72 (2)	87 (2)	97 (2)
15, 12.5, 20 [2]	124 (5)	94 (5)	109 (5)	117 (4)

[1] Numbers in parentheses indicate the number of animals alive at each period.

[2] The first doses were administered for 1 week each; the last dose for the remainder of the growth period. 20 μg. daily was apparently needed for even slow growth.

two of seven animals grown on the lesser amounts of pyridoxine survived 6 months of the deficiency as contrasted with a survival at 8 months of three out of four of the rats previously fed 60 μg. of pyridoxine daily. All of the rats fed the ration containing 2 μg. of pyridoxine per 10 gm. appeared to be normal for at least 8 months.

DISCUSSION

A summary of the maintenance records on the deficient diets (table 4) indicates that an adult rat can be deprived of any one of the B vitamins except thiamine without visible damage for several months. Ultimately, of course, deficiency symptoms develop due to a lack of riboflavin, pantothenic acid, or pyridoxine. If the consumption of pyridoxine has

TABLE 4

Summary: Maintenance of adult rats on diets low in the various B vitamins

MAINTENANCE DIET	PERIOD OF GROWTH	PERIOD SEEMED NORMAL	PERIOD MAINTAINED WEIGHT	PERIOD 50% SURVIVED	DEFICIENCY SYMPTOMS		COMMENTS
					Description	Appeared	
	mo.	*mo.*	*mo.*	*mo.*		*mo.*	
Control	3	12	12	12	None	..	.....
Nicotinic acid- and choline-free	3	12	12	12	None	..	.....
Thiamine-free	1/2	1/2	1/2	1	Poor co-ordination	1	High levels during growth prolonged life
Riboflavin-free	1	2–3	6	8	Listlessness alopecia dermatitis	3 4 4	
Pantothenic acid-free	1	2–3	4–7	5	Poor tonus "Blood-iness"	3 4	High levels during growth did not prolong life
Pyridoxine-free	1–2	3–5	8–10	8[1]	Poor tonus Hypersen-itivity	4 5	High levels during growth markedly prolonged life

[1] Over-all average: Those grown on the high vitamin diet all survived longer than 12 months.

been high during the period of growth, the rat can subsequently survive for at least 12 months on a diet very low in this vitamin. A few preliminary experiments indicate that good survival results when only 2 μg. of pyridoxine per 10 gm. are added to the deficient maintenance diet. In view of the observations cited in the introduction, the present results are sufficiently encouraging to warrant long-term tumor experiments with adult animals fed suboptimal diets.

SUMMARY

1. Rats were raised from weaning to 12 weeks of age on synthetic diets containing 0.1% choline and low, medium, or high amounts of

nicotinic acid, thiamine, riboflavin, pantothenic acid, and pyridoxine. They were then maintained for periods up to a year on a diet containing all of these vitamins or on diets from which one of them had been omitted.

2. On the diet free of thiamine the rats lost weight and died in 3 to 7 weeks. Most of the adults on the other deficient diets survived for many months. Those deprived of nicotinic acid and choline for a year were comparable in appearance, growth, and survival to rats receiving these vitamins.

3. The rats on the diet free of riboflavin lived 7 to 12 months. They soon ceased to grow but appeared normal for about 4 months on the deficient diet, after which characteristic symptoms of deficiency developed.

4. Half of the rats on the diet free of pantothenic acid survived for 5 months. These rats stopped growing in 1 month and lacked muscle tonus in 2 to 3 months. On autopsy hemorrhagic areas were found in the intestinal tract.

5. Rats grown on a diet containing 60 μg. of pyridoxine per 10 gm. of ration subsequently survived for at least 12 months of maintenance on a diet deficient in this vitamin. Those grown on lesser amounts of pyridoxine lived for an average of 8 months. Growth ceased in 1 month and a general atony developed after 3 months more.

6. It is concluded that adult rats can survive in a reasonably healthy state on very low amounts of certain critical vitamins, and that experiments on the therapeutic value of tolerable nutritional deficiencies are feasible.

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THE B VITAMIN CONTENT OF GROATS AND ROLLED OATS [1]

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A survey of the literature indicates a limited amount of information on the B vitamin content of oats. Schultz et al. ('41) studied the thiamine content of twenty-one samples of oats using the fermentation method and found a range of 4.8–10.3 μg. per gram (.48–1.03 mg. per 100 gm.) and an average of 7.2 μg. per gram. Nordgren and Andrews ('41) used the thiochrome method and reported the average thiamine content of six samples of oats as 4.2 mg. per pound (.94 mg./100 gm.). However, the authors claim that this value is probably higher than the nation wide value for oats since the oats were obtained from a station where the thiamine values of wheat and corn were found to be higher than previously reported values. Moir ('42) in a review article has given thiamine values which are distinctly lower. Snell and Strong ('39) determined the riboflavin content of a few samples of oats, but their values are probably too high since in their microbiological method they did not remove interfering fatty acids. Bacharach ('41) in a review article has given a few nicotinic acid values. In this investigation we have assayed several samples of hulled oats (groats) and rolled oats for their thiamine, nicotinic acid, pantothenic acid, riboflavin and pyridoxine content.

EXPERIMENTAL

Samples of hulled oats were obtained from the three commercial mills [2]. We also assayed samples of experimental hand hulled oats obtained from Dr. H. L. Shands of the Department of Agronomy. All samples were ground in the Wiley mill with the medium sieve.

Thiamine was determined by the Hennessy-Cerecedo thiochrome fluorescence method as modified by Hennessy ('42). Samples were prepared as follows: One gram of the powdered oats was digested with 75 ml. 0.1 N H_2SO_4 at 100°C. for 1 hour, and then cooled below

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[2] Cedar Rapids, Iowa; Akron, Ohio; and St. Joseph, Missouri. We are indebted to the Quaker Oats Co. of Chicago, Illinois, for these samples.

50°C. after which 5 ml. of a 2.5 N sodium acetate solution containing 5% clarase were added. It was then kept at 45–50°C. for 1 hour after which it was held overnight in a 37°C. incubator. After filtering, an aliquot portion was adsorbed on a column of Decalso, eluted with 25% KCl in 0.1 N HCl and oxidized with a 0.03% $K_3Fe(CN)_6$ solution in 15% NaOH. The fluorescence of the thiochrome formed was read in the Coleman photofluorometer. Values are expressed as mg. of thiamine hydrochloride per 100 gm.

Pantothenic acid was determined by the microbiological method of Strong et al. ('41). Samples were prepared as follows: 0.5 gm. of powdered sample was suspended in 75 ml. distilled water, autoclaved at 15 pounds pressure for 15 minutes and finally diluted to the necessary volume. Values are expressed as mg. calcium pantothenate per 100 gm.

Riboflavin was determined by the microbiological method of Snell and Strong ('39) as modified by Strong and Carpenter ('42). Samples were prepared as follows: 50 ml. of 0.1 N HCl were added to 5 gm. of powdered sample, autoclaved at 15 pounds pressure for 15 minutes, cooled and then 3.3 ml. of 2.5 molar sodium acetate was added. The mixture was then adjusted to a volume of 100 ml., filtered through Whatman no. 40 filter paper, and 50 ml. aliquot of the clear filtrate adjusted to pH 6.8–7.0 and made up to 100 ml. Trial runs showed that this procedure was adequate for the removal of interfering substances since subsequent extraction with ether gave similar results. Values are expressed as mg. riboflavin per 100 gm.

Nicotinic acid was determined by the microbiological method of Snell and Wright ('41) as modified by Krehl et al. ('43). In this modification higher concentrations of glucose, sodium acetate, and cystine are added to the basal medium. Samples were prepared as follows: 25 ml. of 1 N NaOH were added to 1 gm. of powdered sample, autoclaved at 15 pounds pressure for 15 minutes, neutralized and diluted to the necessary volume. Values are expressed as mg. nicotinic acid per 100 gm.

Pyridoxine was determined by the microbiological method of Atkin et al. ('43). This method employs the response of a strain of yeast to pyridoxine, the response being measured as turbidity readings on the Evelyn colorimeter. Samples were prepared as follows: 1 gm. of powdered sample was suspended in 180 ml. 0.44 N H_2SO_4, heated at 20 pounds pressure for 1 hour in the autoclave, cooled, neutralized to pH 5.2, and then adjusted to 200 ml. Various levels of the unknown solution were added to 125 ml. Erlenmeyer flasks which contained 5 ml. of the pyridoxine free basal medium and then sufficient distilled

water was added to make a total volume of 9 ml. The flasks were then inoculated with 1 ml. each of the yeast inoculum. They were then kept in a 30°C. incubator for 18 hours where they were shaken two or three times during the period of incubation. This procedure gave consistent results and recoveries. Values are expressed as mg. pyridoxine hydrochloride per 100 gm.

RESULTS AND DISCUSSION

In table 1 are shown the results of analysis of the commercial oats. The table is arranged in pairs giving the B vitamin content of hulled oats (groats) and of the rolled oats prepared from these groats. It

TABLE 1

Vitamin content of groats and rolled oats.

MILL		THIAMINE	NICOTINIC ACID	PYRIDOXINE	PANTOTHENIC ACID	RIBOFLAVIN
		mg./100 gm.	*mg./100 gm.*	*mg./100 gm.*	*mg./100 gm.*	*mg./100 gm.*
Cedar Rapids, Iowa	Groats 1	0.686	0.96	0.130	1.39	0.127
	Rolled oats 1	0.672	0.90	0.100	1.35	0.120
Cedar Rapids, Iowa	Groats 2	0.612	0.78	0.104	1.58	0.137
	Rolled oats 2	0.582	0.72	0.099	1.47	0.130
Cedar Rapids, Iowa	Groats 3	0.636	0.84	0.104	1.82	0.135
	Rolled oats 3	0.636	0.86	0.104	1.75	0.133
Akron, Ohio	Groats 4	0.734	0.69	0.104	1.26	0.126
	Rolled oats 4	0.672	0.66	0.104	1.14	0.119
Akron, Ohio	Groats 5	0.690	0.82	0.119	1.65	0.150
	Rolled oats 5	0.668	0.78	0.098	1.65	0.141
Akron, Ohio	Groats 6	0.746	0.88	0.116	1.17	0.129
	Rolled oats 6	0.652	0.86	0.122	1.09	0.122
St. Joseph, Missouri	Groats 7	0.800	0.90	0.130	1.75	0.155
	Rolled oats 7	0.784	0.88	0.135	1.67	0.146
St. Joseph, Missouri	Groats 8	0.827	0.96	0.148	1.62	0.167
	Rolled oats 8	0.790	0.96	0.149	1.62	0.159
St. Joseph, Missouri	Groats 9	0.830	0.92	0.122	1.60	0.135
	Rolled oats 9	0.812	0.94	0.120	1.60	0.130
AVERAGE VALUES						
Cedar Rapids, Iowa	Groats	0.645	0.86	0.113	1.60	0.133
	Rolled oats	0.630	0.83	0.101	1.52	0.128
Akron, Ohio	Groats	0.723	0.80	0.113	1.36	0.135
	Rolled oats	0.664	0.77	0.108	1.29	0.127
St. Joseph, Missouri	Groats	0.819	0.93	0.133	1.69	0.152
	Rolled oats	0.795	0.93	0.138	1.63	0.145

can be seen that there is only a slight loss in the pyridoxine, thiamine, riboflavin, nicotinic acid and pantothenic acid content in the preparation of rolled oats from groats and in many cases this apparent loss is within the limits of the experimental error.

From table 1, it can also be seen that there is a tendency for the groats and rolled oats from the St. Joseph, Missouri, mill to be higher in all the B vitamins determined. This may be due to variation in oats obtained from different regions.

TABLE 2

Vitamin content of experimentally hand-hulled oats.

VARIETY	THIAMINE	NICOTINIC ACID	PYRIDOXINE	PANTOTHENIC ACID	RIBOFLAVIN
	mg./100 gm.	*mg./100 gm.*	*mg./100 gm.*	*mg./100 gm.*	*mg./100 gm.*
1. States Pride Ped. 7	1.03	1.35	0.163	1.75	0.137
2. Vieland	1.20	1.10	0.172	2.35	0.144
3. Tama	1.04	1.05	0.139	2.50	0.151
4. Erban	1.21	1.25	0.160	3.50	0.148
5. Marion	1.32	1.20	0.160	2.70	0.143
6. X215–19	1.17	0.725	0.152	2.20	0.126
7. X219–1–2	1.22	1.08	0.142	2.75	0.182
8. X219–1–3	1.13	0.825	0.257	2.40	0.250
9. X220–13	1.23	0.925	0.196	2.45	0.165
10. X254–1–1	1.22	1.15	0.207	2.85	0.155
11. X254–6	1.00	1.50	0.313	2.20	0.220
12. X306–1	1.17	1.22	0.169	3.20	0.196
13. C. I. 3664	1.22	1.00	0.203	2.50	0.145
14. C. I. 4170	1.00	0.95	0.300	1.70	0.133
AVERAGE VALUES	1.15	1.095	0.195	2.50	0.164

In table 2 are summarized the values obtained on the experimentally produced oats. It is evident that these hand hulled oats are distinctly higher in the B vitamins than those summarized in table 1. The values for thiamine show the largest and those for riboflavin the smallest differences. These variations are not surprising since the experimental oats were produced on plots that were well fertilized and were harvested under controlled conditions. The hulls were removed by hand which allowed not only selected samples but may have allowed a greater retention of vitamins. It is also interesting to note that the experimental oats were made up of several varieties and that no one variety was consistently higher than the others in all the vitamins.

While the results presented in this paper give a general picture of the B vitamin content of oats it should be emphasized that samples collected from other areas of the country and grown under other environ-

mental conditions may differ somewhat in composition. The methods used for the estimation of the vitamins were very satisfactory and almost identical results were obtained when the same sample was analyzed at different periods. However, some difficulty was encountered when the thiamine determinations were made on the same original sample but ground under slightly different conditions. This difficulty is apparently encountered only in the case of oats, and until means are found for completely eliminating this variation it may be necessary to allow a 10% differential in the thiamine results.

SUMMARY

The thiamine, nicotinic acid, pantothenic acid, riboflavin and pyridoxine content of about thirty samples of commercial oat groats and rolled oats, and experimentally hand-hulled oats, are given.

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STUDIES OF THE COMPARATIVE NUTRITIVE VALUE OF FATS

II. THE COMPARATIVE COMPOSITION OF RATS FED DIFFERENT DIETS [1]

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In an earlier study from this laboratory, no significant differences were noted in the rate of growth of white rats over a 12-week period following weaning (Deuel, Movitt, Hallman and Mattson, '44) when different fats were added to a basal diet of mineralized skimmed milk powder and vitamin supplements, in an amount of 29.4%. The fats tested were butter and margarine, and corn, cottonseed, olive, peanut, and soybean oils. The present study was designed to determine whether any differences obtained in the composition of the rats which had received the different diets over a period of 12 weeks.

The present tests were made on 102 rats of series II; the details of the experimental procedure are described earlier (Deuel, Movitt, Hallman and Mattson, '44). After 12 weeks on the various diets, the animals were anesthetized with sodium amytal, weighed, the stomach and intestines were removed and weighed, and the carcass was ground to a homogeneous mass by passing through a meat grinder three times. The weight of the rat and the water content of the aliquot were corrected for the amytal injected.

Water was determined by drying aliquot samples to constant weight in the vacuum oven at approximately 60°C. The dried tissue was then extracted for 16 hours in a Soxhlet extractor with diethyl ether. After removal of the ether, the tissue was reground in a Wiley mill and the dried, defatted powder used for determination of nitrogen by the Kjeldahl method, and of ash. Calcium was determined on the ash by

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titration of the oxalate with $KMnO_4$ as described by Hawk and Bergeim ('37). Carbohydrate was estimated by difference.

The results are summarized in table 1.

The composition of the rats is quite uniform in the different groups but varies with the sex of the animals. In no case was any statistically valid difference found between groups. The values for protein vary in the males between 17.8 and 16.2% and in the females between 17.0 and 15.6%. These are somewhat lower than other values in the literature, i. e., 18.75 (Terroine et al., '23, '24), 18.83 (Light et al., '34), 20.5 (Truszkowski, '26), and 21.1% (Chanutin, '30). The ash content varied in the males between 2.87 and 3.16% and in the females between 3.22 and 3.28%. These values agree well with the 3.38% reported by Light et al. ('34) and the 3.10% obtained by Buckner and Peter ('22) for 16-week-old rats.

On the other hand there is a definite sex difference in the composition of the rats. In general, the males have a higher water and protein content and a lower percentage of lipid, ash and calcium. Although these differences are usually not significant statistically when the comparisons are made for the animals on a single diet, it is to be noted that these variations are found in every average with the various components mentioned above (except in one case with the percentage of water). Based on a P value of 0.01, the "t" values [2] for the variations in ash and calcium are significantly different between the male and female rats on the corn oil, cottonseed oil, margarine and peanut oil diets. When the statistical evaluation is made on the average analyses of all the males and females, the differences are significant for protein, ash and calcium and practically so for water and lipid. The sex variation in calcium content is in agreement with earlier observations of Lanford, Campbell and Sherman ('41). Although the absolute values varied with the age of the rats, the percentage of calcium of the 90-day-old rats on several diets was as follows: diet 13 (0.34% Ca) males 0.899, females 1.066; diet 132 (0.48% Ca) males 0.968, females 1.126; diet 133 (0.64% Ca) males 1.000; females 1.187. These values are higher than obtained in our tests (males 0.911, females 1.028) where the diet had 0.88% Ca. However, our diets contained approximately 30% of fat whereas in the above tests the diets had one-third skimmed milk powder and two-thirds whole wheat flour, the increased calcium being supplied with the last two diets by the addition of $CaCO_3$.

[2] In all cases the "t" value exceeded that required for a "P" value of 0.01 (one chance in 100 that the results occurred by chance). This method of statistical evaluation is more accurate when the number of cases is under ten (Fisher, '36).

TABLE 1

The average composition of the tissues of eviscerated rats which had previously received a mineralized skimmed milk diet with different fats for 12 weeks after weaning at 3 weeks of age.

SEX	BODY WEIGHT		COMPOSITION ON WET BASIS IN PER CENT[1]					
	Normal	After evisceration	Water	Protein	Lipid	Carbohydrate	Ash	Ca
	gm.	*gm.*						*mg./%*
Male (51 rats)	254.8	237.0	58.2±0.4 (59.8—56.8)	17.3±0.2 (17.8—16.2)	18.1±0.5 (20.5—16.2)	3.38±0.37 (4.01—2.64)	2.95±0.03 (3.16—2.82)	911±11 (958—891)
Female (51 rats)	171.2	158.3	56.8±0.4 (57.8—54.7)	16.4±0.2 (17.0—15.6)	20.1±0.6 (23.3—19.2)	3.53±0.12 (3.99—3.12)	3.26±0.04 (3.30—3.22)	1028±16 (1060—1004)
M.D.: S.E.M.D.[2]			2.45	3.10	2.70	0.40	6.20	6.03

The values in parentheses are the limits of the means of the groups on the various diets.

[1] Including the standard error of the means calculated as follows: $\sqrt{\frac{\epsilon d^2}{n}} \Big/ \sqrt{n}$

[2] Mean Difference: Standard Error of Mean Difference. When this exceeds 3, the results are considered significant. The comparisons in each case are between the same components in the male and female rats.

SUMMARY

No differences were found in the composition of rats which had received diets of mineralized skimmed milk powder and vitamin supplements together with butter or margarine fat, corn, cottonseed, olive, peanut or soybean oils over a 12-week period. However, it was noted that male rats have a slightly higher water and protein content and a lower lipid, ash and calcium content than the females.

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STUDIES OF THE COMPARATIVE NUTRITIVE VALUE OF FATS

III. THE EFFECT OF FLAVOR ON FOOD PREFERENCE [1]

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Several factors are involved in the regulation of appetite. Probably foremost of these is the instinctive preference of animals for required nutrients which is exercised even to a limited extent by the young animal (Carlson, '16). The selection of salt solution (Richter, '36) and a low potassium diet (Zwemer and Truszkowski, '36) by adrenalectomized rats as well as the choice of calcium lactate solution (Richter and Eckert, '37) by parathyroidectomized rats are instances of such phenomena. Rats will choose qualitatively and quantitatively the protein diets enabling the best growth (Osborne and Mendel, '18) as well as the ones containing adequate vitamins A and undifferentiated B (Mitchell and Mendel, '21). Richter, Holt and Barelare ('37, '38) found that rats would select a diet adequate for normal growth and reproduction from a number of purified foodstuffs simultaneously and separately available. Although flavor may play an important role in the determination of appetite, it is operative only when the diets are reasonably complete; it is entirely ineffective when deficiencies in the diet exist. Thus, Cowgill, Deuel and Smith ('25) were unable to stimulate the appetite of B-deficient dogs with beef extract although moderate doses of a vitamin B concentrate restored the dog's appetite for the bland synthetic diet.

However, on complete diets, flavor may play a role in food consumption with rats as well as with man. The aversion of rats to diets containing rancid fats is well known; the recent report of Clausen, Barnes and Burr ('43) emphasizes the relationship of rancidity to the

[1] This work was carried out under a research grant from The Best Foods, Inc. The authors wish to acknowledge the helpful advice of Prof. Anton J. Carlson of the University of Chicago, of Prof. Arthur W. Thomas of Columbia University and of Dr. H. W. Vahlteich of The Best Foods, Inc. during the course of the experiments.

destruction of the B vitamins, particularly when mixed with the food in pure form. In the experiments of Boutwell et al. ('41) where a comparison is made of the growth of weanling rats on mineralized skimmed milk homogenized with butterfat or with vegetable oils, the greater growth of the rats receiving the butter was associated with a greater consumption of this diet. Although admitting that the greater growth of the butter group was associated with a higher food intake, these authors state that increased appetite with the consequent augmented food consumption is an attribute of a superior food.

In earlier papers (Deuel et al., '43, '44) we have reported growth experiments on a diet containing mineralized skimmed milk powder mixed with butter, margarine, corn, cottonseed, olive, peanut or soybean oil. Although no differences in growth were noted at 3 weeks as reported by Schantz et al. ('40), somewhat greater growth obtained in the butter group at 12 weeks with the females although the differences were not noted in the experiments on male rats. Inasmuch as there was some evidence that the greater growth was the result of a larger food consumption, the present study was undertaken to determine whether rats prefer a diet containing butter to one containing other fats. Secondly, after this preference was demonstrated, it was next important to determine whether the preference for the butter diet was an instinctive one for the fat itself or whether it was related to the components in the butter responsible for its distinctive flavor. Tests were then made on the comparative food intake when rats were offered two identical diets one of which was flavored with diacetyl or a commercial butter flavor and one unflavored. The quantity of diacetyl preferred by the rat was also determined.

METHODS

The experiments were made on 21-day-old rats from our stock colony over periods of 3 to 12 weeks. The mothers were on the stock diet prior to the weaning of the litter. The rats were kept in separate cages and the two foods being tested were offered in identical food cups. When the spilling of the food exceeded the difference in apparent food consumption of the two diets, the experiment was discarded.

In the experiments which were carried out over 12 weeks, another procedure was adopted. Two of the individual cages were connected; both had water bottles, and one diet was placed in one cage and the other diet in the second cage. If any spilling occurred, any food so lost could be weighed and correction made. The position of the food cups was reversed daily.

The diet was the same as that described earlier (Deuel et al., '44). Diacetyl [2] and butter flavor [3] obtained commercially were used.

RESULTS

In the first series of tests, the butter diet and one of the diets containing the other fats (corn, cottonseed, olive, peanut or soybean oils or margarine) were offered simultaneously. These results are summarized in table 1.

In every series of tests the butter diet was consumed in the larger amount. The maximum difference was found in the case of the animals receiving also the soybean oil diet where the butter diet was taken in a proportion of more than two to one. The preference for the butter diet is also evident in the number of weeks where a greater amount was consumed. On this basis the highest choice for the butter diet was evident when margarine was also offered, the butter diet being preferred in a proportion of over 6 to 1 compared with the margarine diet.

That the preference for the butter diet is related to its flavor, is indicated by the tests recorded in table 2. This gives the comparison in food intake and diet preference on unflavored peanut or margarine diets and on similar diets flavored with diacetyl or with commercial butter flavor. The level of the flavor preferred is also shown.

DISCUSSION

The experiments reported in table 1 demonstrate that in the majority of instances rats prefer a complete diet where the fat is butter to one where this fat is replaced by vegetable fats. The total score was 106 cases where the butter diet was consumed in greater quantity to 55 where the vegetable fats were preferred. When compared with each fat, the butter was preferred. The results range from a practically even choice with olive oil (13 to 12) and cottonseed oil (15 to 13) to a more marked preference with corn oil (17 to 11) and a decided variation with the other three fats (peanut oil 18 to 10, soybean oil 23 to 6, and margarine 20 to 3). These differences are more clearly evident, however, on the basis of food consumption where in every case the amount of butter diet ingested is definitely greater than that of the vegetable fat. These vary from an amount of 24.3 to 20.6 gm. per rat per week with the butter and corn oil diets respectively to values of more than 2 to 1 in favor of the butter with the butter and soybean oil groups (29.0 gm. to 13.5 gm.).

[2] Prepared by Larkin and Co., Buffalo, N. Y.

[3] Verley B F A obtained from Verley Products Corporation, 1621 Carroll Ave., Chicago, Ill.

TABLE 1

The average food consumption of rats and their dietary preference when a butter diet (B) and one containing a vegetable fat (A) were offered simultaneously over a 3-week period after weaning.

DIET COMPARED WITH BUTTER DIET	AVERAGE FOOD CONSUMPTION PER RAT PER WEEK								DIET PREFERENCE							
	1st week		2nd week		3rd week		AVERAGE		1st week		2nd week		3rd week		TOTAL	
	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*								
Corn oil	13.1 (9	13.8	25.7 (8)	24.6	35.4 (8)	24.2	24.3 (25)	20.6	5	4	6	4	6	3	17	11
Cottonseed oil	15.5 (10)	13.0	25.4 (10)	16.8	30.7 (7)	27.4	23.2 (27)	18.1	5	5	5	5	5	2	15	13
Margarine	22.8 (9)	11.4	26.0 (8)	15.6	38.6 (7)	23.3	28.5 (24)	16.2	8	1	7	1	5	1	20	3
Olive oil	9.0 (7)	17.4	21.9 (8)	17.6	36.7 (9)	19.2	23.7 (24)	18.1	2	7	4	4	7	1	13	12
Peanut oil	15.7 (10)	11.7	25.5 (8)	14.0	29.6 (10)	25.3	23.5 (28)	17.2	6	4	6	2	6	4	18	10
Soybean oil	19.4 (10)	9.5	30.7 (9)	12.1	37.3 (10)	18.9	29.0 (29)	13.5	7	3	8	1	8	2	23	6

The figures in parentheses are the number of experiments upon which the average is based.

TABLE 2

The comparative food intake and preference of rats for diets flavored with diacetyl or commercial butter flavor to similar unflavored diets.

FLAVOR	BASAL DIET	FLAVOR ADDED [1]		NO. OF RATS	WEEKS OF TESTS	TOTAL NO. OF TESTS	FOOD EATEN		DIET PREFERENCE		
		Diet 1	Diet 2				Diet 1	Diet 2	1	2	Tied
		p.p.m.	*p.p.m.*				*gm.*	*gm.*			
Diacetyl	Margarine	0	4	4	2	8	5.0	21.8	0	8	0
		4	8	4	3	11	25.7	14.1	8	3	0
		8	16	4	3	9	24.1	13.3	7	2	0
		16	24	3	3	8	21.4	19.9	3	5	0
	Peanut	0	4	4	3	12	9.2	34.0	0	12	0
		4	8	4	3	11	16.1	18.5	5	6	0
		8	16	4	3	10	15.6	18.3	6	4	0
		16	24	4	3	10	24.9	9.8	8	2	0
Diacetyl (1) and butter flavor (2)	Margarine	4	4	10	3	30	20.7	27.9	7	22	1
Butter flavor	Margarine	0	4								
Group I [2]				8	12	92	31.5	41.3	19	69	4
Group II				4	12	44	40.8	38.9	21	21	2
	Peanut	0	4								
Group I [2]				8	12	95	27.3	41.3	18	77	0
Group II				4	12	48	34.5	36.7	22	25	1

[1] Parts per million in fat of diet.

[2] The division into groups is based solely on food preference in an experiment on twelve animals carried on simultaneously.

The preference for the butter diet would seem to be because of the unique flavor which this fat possesses. If this flavor is similar in rat-milk fat to cow-milk fat, the rat would become conditioned to this taste during the period of suckling. The remarkable delicacy of this response is indicated by the fact that it can be brought about by the addition of commercial butter flavors in an amount of 4 parts per million parts of fat or 1.3 parts per million parts of diet. When fed in this concentration with margarine or peanut oil diets, the flavored diets were taken in larger amounts in all twenty tests. The average food consumed of the unflavored and flavored diets leaves no doubt about the preference of the rats (margarine diet, 5.0 to 21.8 and peanut oil diet, 9.2 to 34.0 gm.). With the margarine diet, higher amounts are less acceptable to the rat than the lower concentrations of diacetyl; on the other hand, no preference was noted with the peanut oil diets except at the highest levels where 16 parts per million were decisively preferred to 24 parts per million. Further, it has been shown that the commercial butter flavor in which diacetyl is present to only 12% is a flavor even more acceptable to the rat, being preferred twenty-two times to seven for the diacetyl diet. A laboratory prepared butter flavor containing monobutyrin, butyric acid and diacetyl to simulate commercial butter flavor gave similar results.

The food consumption with the diet having the butter flavor averages 40% higher than in the diacetyl-flavored diet. Also apparently the rats prefer this at the lower level (4 parts per million) than at one which would give 4 parts of diacetyl (33 parts per million).

These differences in food intake are quite marked over an entire 12-week period. In each of twelve experiments carried out on margarine or peanut oil diets flavored with commercial butter flavor or unflavored, eight rats in each group consistently preferred the flavored diet, the total score being 69 to 19 (with 4 tied) in the margarine diet, and 77 to 18 with the peanut oil diet. With respect to the average food consumption of the two types of each diet the same differences are evident, the flavored and unflavored diets being consumed on an average as follows: margarine diet, 41.3 and 31.5 gm. per rat per week; peanut oil diet, 41.3 and 27.3 gm. With four rats in each group no consistent differences in food intake were to be found. The total score was 21 and 21 (with 2 tied) with the margarine tests, and 25 to 22 (with 1 tied) with the peanut oil diets. Also the average food consumption of the flavored and unflavored diets was practically identical in these two series of tests. It is important that in these groups of rats which were

indifferent in their choice of diets, in no case was there evidence of preference for unflavored food.

The preference of rats for a butter diet over one containing vegetable fats which Boutwell et al. ('41) and we have demonstrated appears to be related to the flavor, part of which is dependent on its diacetyl content. There is no evidence that diacetyl or butter flavor possesses any properties which would include them in the category of vitamins. Not only may they be manufactured in the adult lactating female, but also as far as known they do not possess any physiological function in the animal body.

SUMMARY

Rats prefer a diet of mineralized, vitamin fortified skimmed milk powder containing butter to one where the fat is corn, cottonseed, olive, peanut or soybean oil, or margarine. The preference is apparently associated with flavor. The animals universally preferred a margarine or peanut oil diet containing 4 parts per million of diacetyl to an unflavored diet, also, this low level in general is preferred to 8, 16 or 24 parts per million of diacetyl. Commercial butter flavor is favored over diacetyl. It is concluded that flavor may play an important role in determining food consumption of diets which are satisfactory from a nutritional standpoint. Although this would not be consistently demonstrated in all animals with flavored and unflavored margarine or peanut oil diets, two-thirds of the rats consistently chose in a ratio of 4 to 1 the flavored diet while no preference was evident in the remaining animals between the two diets. In no case was there a consistent preference for the unflavored diet.

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STUDIES ON THE URINARY EXCRETION OF RIBOFLAVIN[1]

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ONE FIGURE

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It is generally agreed that riboflavin is an essential constituent of the human dietary but there is not such general agreement concerning the amount necessary and the symptoms produced by a deficiency. In view of this an objective laboratory test which will give an index to the status of the individual with respect to riboflavin becomes of considerable importance. Since riboflavin is water soluble it might be expected that it would appear in the urine and that the amount in this fluid might give an index to the supply available in the body. Three other factors also point in this direction: (1) the urine contains only free riboflavin, (2) riboflavin has the property of fluorescence, and (3) the compound and therefore the fluorescence are readily destroyed quantitatively by exposure to light. The latter two facts can be made the basis for a simple and rapid method of determination.

Several investigators have in consequence determined the urine content of riboflavin (Ferrebee, '40; Williams et al., '43; Swaminathan, '42; Strong et al., '41; Helmer, '37; Emmerie, '36; Sebrell et al., '41; Axelrod et al., '41; Najjar and Holt, '41; Najjar, '41). Four conclusions can be drawn from the results presented in these papers: (1) the 24-hour excretion of riboflavin in the urine varies rather widely among normal individuals on a presumably normal diet, (2) only a fraction of that ingested is excreted, (3) increased intake leads to increased excretion and vice versa, and (4) figures of the same order of magnitude are obtained in widely scattered parts of the world.

The variability in the urinary output has led to the conclusion (Gyorgy, '42) that determinations of riboflavin in the urine are of little if any value in detecting incipient or long standing deficiency. However, in view of the ease with which the determinations can be carried out, it seemed to us that before the idea was discarded further

[1] Aided by a grant from the Nutrition Foundation, Inc.

attempts should be made to test the adequacy of laboratory determinations as an index of riboflavin status.

METHODS

We have used the method and in general the procedure of Najjar ('41). The direct method has been used in all cases and has proven quite satisfactory in our hands.[2] When two extractions are carried out, this method allows a reading after the destruction of the riboflavin by light, and therefore, a blank reading on each sample. Complete disappearance of the riboflavin was checked for each batch of urine extracts by carrying a standard amount of riboflavin through the procedure including the exposure of an aliquot to sunlight. To date we have carried out such determinations on 658 urine specimens. The samples have been obtained from several widely separated groups and in collecting them we have had two ends in view: (1) to ascertain whether the determination of the urinary riboflavin provides reliable information as to the status of the individual for this vitamin, and (2) to determine whether the average person in this locality has adequate amounts of riboflavin in his diet.

Determinations have been carried out on samples from laboratory workers, hospital and private patients, medical students, men called by a local draft board for physical examination, and a large group of individuals of various ages and economic status from a rural area of Fulton County. In some instances 24-hour urine samples were obtained but usually only fasting morning specimens were analyzed as recommended by Najjar. Where it was not possible to obtain morning samples, hourly specimens were collected and an attempt made to ascertain whether the most recent meal contained substances high in riboflavin such as milk and liver.

RESULTS

The results obtained are shown in figure 1. When these figures were collected the question arose as to how they should be expressed. The 24-hour excretion value seems to have only limited significance (Najjar and Holt, '41) and the alternatives were to calculate either the hourly excretion or the amount per milliliter of urine. When our results were examined, there seemed for the most part to be better correlation between the riboflavin found per unit of volume than per unit of time. Table 1 serves to illustrate the type of result obtained when both

[2] Readings were made on a Pfaltz and Bauer, Model B, Fluorophotometer.

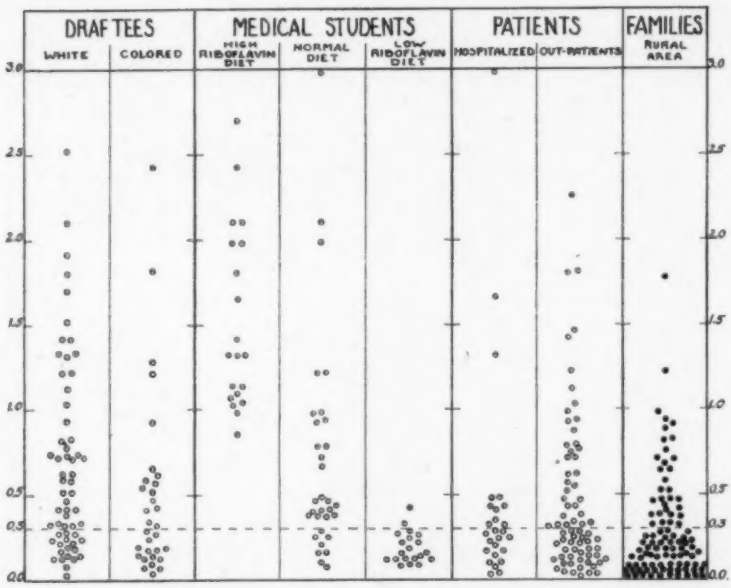

Fig. 1 Riboflavin excretion. Solid circles represent fasting morning samples while open circles represent casual hourly samples.

methods of calculation are applied to the values from four subjects selected from our series. It will be noted that the hourly urine volumes vary widely, in one case (M.M.) nearly ten-fold and the riboflavin excretion in micrograms per hour falls within about the same limits; however, when the output of riboflavin is expressed in micrograms per milliliter of urine, the values for the two urine samples are identical. It is also of interest that the urine specimens in this instance were collected some 9 weeks apart.

From our data, of which table 1 gives only a sample, we became convinced that the excretion per milliliter of urine is the more constant

TABLE 1

Comparison of fasting riboflavin excretion per hour and per milliliter. Illustrative data.

SUBJECT	S.G.		M.M.		M.W.		I.N.	
Date	8/4	8/5	8/25	12/1	9/22	9/29	8/17	8/22
Urine for hr., ml.	45	130	24	208	202	44	207	94
Riboflavin, μg./hr.	15.3	43.2	1.4	12.5	114.4	25.0	26.3	14.1
Riboflavin, μg./ml.	0.34	0.33	0.06	0.06	0.56	0.57	0.12	0.15

figure and in subsequent work we have used this method of expressing our results.

Our next concern was in establishing a level for urinary riboflavin which should represent a division between the excretion resulting from an adequate intake and that resulting from a diet deficient in this substance. Swaminathan ('42) found that with an intake of 1.2–1.5 mg., 25–30% of the ingested riboflavin appeared in the urine. On a dietary level of 2–3 mg. per day, Sebrell et al. ('41) report that 28–48% was excreted by the kidney. The values of Williams et al. ('43) also fall within this range. A typical individual, then, with a riboflavin intake of 2–3 mg. per day which is the amount advocated by the Committee on Food and Nutrition of the National Research Council, might be expected to have a urinary output in each 24 hours of 500–1000 μg. with an average of perhaps 800 μg. For an average urine volume of 1000 ml. this would be 0.8 μg. per milliliter; for a volume of 1500 ml., 0.53 μg. When it comes, then, to fixing a value for riboflavin excretion per unit volume above which an individual will be considered adequately supplied with riboflavin and below which he will be considered deficient, it seems that perhaps 0.53–0.8 μg. per milliliter should be the figure selected. Even though we have for the most part dealt with fasting samples, if the organism is adequately supplied with riboflavin, this level of excretion should persist through the 15 hours of fasting; however, we have felt that for our purposes it would probably be better to set the figure considerably lower and thus avoid the charge of telling an individual he was suffering from a deficiency when in reality he was merely somewhat below the optimum nutrition level. Therefore, for the interpretation of our results we have set 0.3 μg. per milliliter as the arbitrary level below which a person will be considered deficient in riboflavin. Further support for this may be seen in figure 1 where it is shown that a liberal intake of riboflavin led with a single exception to an excretion of 0.9 μg. or more per milliliter. A low intake, on the other hand, resulted with a single exception again in an excretion of 0.3 μg. per milliliter or less.

We have also in several instances carried out analyses on a single morning hourly sample collected separately from that for the remaining 23 hours. For the most part the micrograms of riboflavin per milliliter in the hour sample agreed well with the corresponding figure for the larger sample. This is further justification for considering the micrograms per milliliter of riboflavin in a fasting morning specimen as an index of the body's supply.

There is a further step which has been advocated in assaying the status of an individual with respect to nutrient materials and some attempt has been made to apply it to riboflavin. This is the so-called saturation or load test and we have carried out a number of such tests in connection with the determinations on fasting samples referred to above. The results of these analyses are shown in table 2. We felt

TABLE 2

Riboflavin saturation tests.

SUBJECT NO.	RIBOFLAVIN ADMINISTRATION		RIBOFLAVIN EXCRETION						
	Route	Amount in μg.	Fasting μg. per ml.	Percentage of intake during				Total	
				1st hr.	2nd hr.	3rd hr.	4th hr.	Amount in μg.	Per cent of intake
13	I.M.[1]	1000	0.00	13.8	5.7	1.5	0.0	209.9	21.0
19	Orally	1620	0.00	9.45	0.39	11.72	7.07	463.96	28.6
8	I.M.	1000	0.045	6.9	2.3	6.5	3.2	189.1	18.9
13	I.M.	1000	0.10	8.22	1.29	3.45	1.09	140.5	14.05
21	I.M.	1500	0.115	9.23	4.50	2.05	1.30	256.6	17.1
17	I.M.	1500	0.15	0.69	1.32	2.50	4.28	132.01	8.8
8	I.M.	1000	0.18	6.2	6.2	1.8	4.0	181.8	18.2
2	I.M.	1090	0.21	10.0	7.5	3.7	1.7	252.2	22.9
4	Orally	880	0.23	24.0	7.2	5.4	6.11	376.4	42.8
10	I.M.	1000	0.25	27.4	2.1	6.6	4.4	405.4	40.5
15	I.M.	1500	0.26	18.20	3.45	3.93	3.10	430.25	28.7
22	I.M.	1750	0.26	19.3	10.62	6.53	5.20	730.5	41.74
8	I.M.	1000	0.28	6.1	5.08	5.95	0.61	177.44	17.74
5	Orally	768	0.40	37.9	16.1	13.2	10.1	588.7	76.4
21	I.M.	1500	0.406	9.67	6.36	2.87	1.99	313.4	20.9
3	I.M.	1250	0.41	15.12	9.20	12.72	3.72	501.7	40.76
16	I.M.	1000	0.47	17.83	12.4	6.36	6.83	434.2	44.42
3	Orally	1230	0.54	16.0	11.5	6.4	1.9	441.4	35.8
14	I.M.	1000	0.58	17.5	10.1	5.2	2.3	351.9	35.2
19	I.M.	1620	0.59	11.39	9.10	6.12	5.33	517.5	31.9
1	Orally	1250	0.64	21.20	13.15	5.76	3.39	544.4	43.5
9	I.M.	750	0.64	28.2	20.9	13.2	15.0	579.6	77.3
17	I.M.	1500	0.67	6.14	4.76	4.40	0.54	237.67	15.7
6	I.M.	1200	0.76	12.1	14.5	21.8	11.5	719.8	59.9
6	I.M.	1200	0.80	16.7	17.5	7.7	9.1	612.3	51.0
7	I.M.	1340	0.82	22.1	15.7	7.8	7.6	715.3	53.4
9	I.M.	870	0.83	27.5	32.2	19.2	0.7	691.8	79.6
6	Orally	1200	0.91	41.1	18.6	13.1	8.6	977.4	81.4
9	Orally	870	0.99	40.5	21.5	21.5	17.12	880.1	100.5
11	I.M.	500	1.04	49.5	16.8	9.3	7.6	415.9	83.2
12	I.M.	750	1.15	30.7	21.7	8.9	9.7	532.3	71.0
1	Orally	1168	1.2	26.9	11.0	4.8	3.7	542.0	46.4
20	I.M.	1000	1.77	19.48	10.06	7.95	6.35	438.4	43.8

[1] Intramuscularly.

that Najjar's ('41) suggestion of a relatively small test dose had merit and we have therefore adhered to his practice of giving 0.016 mg. per kilogram of body weight. It seemed to us that intravenous administration, which has been used by some investigators, is a decidedly unphysiological method and, therefore, we have for the most part used the intramuscular route.[3] It will be noted that for the most part the same conclusion as to the riboflavin status of the individual was reached from the saturation test (assuming that figures below 35% excretion represent a deficiency) as from the analysis of a fasting sample. Of thirteen subjects with a fasting level less than 0.3 μg. per milliliter, only three excreted more than 35% of the administered riboflavin; while in the group of twenty whose initial excretion was above 0.3 μg. per milliliter, only three showed less than 35% excretion.

The coefficient of correlation between the fasting excretion expressed in micrograms per milliliter and the per cent of the test dose recovered within 4 hours is 0.61. This indicates a good degree of probability that the second figure depends on the first and that, in most instances, a correct conclusion will be drawn from an examination of only a fasting morning specimen. This saves considerable time as compared with the more laborious and time-consuming saturation test.

Examination of figure 1 will show that on the basis discussed above, a deficiency of riboflavin is a rather common finding and occurs to a greater or lesser degree in all the groups studied. Of the group of medical students, 25% were deficient as compared with 30% of the group of white draftees of approximately the same age but of differing economic status. Also, it is interesting that of a corresponding group of colored draftees, 50% showed a deficiency. A considerably higher percentage of the hospital patients had low values but that might have been anticipated because they were a highly selected group. Of the persons selected at random in the rural area about 65% showed a deficiency indicating, we believe, that a low intake of riboflavin is widespread throughout the general population in this area. We are well aware that single determinations of riboflavin excretion in the urine, such as we have carried out on many of our subjects, represent only isolated instances of a continually changing pattern. Subsequent analyses might have resulted in a different impression. However, when the figures are obtained from a rather large group of individuals, we feel that the finding of considerable deficiency is valid for the group

[3] We are indebted to the Winthrop Chemical Company, Inc., Department of Medical Research, for the riboflavin (Flavaxin Soluble, Niphanoid) used in these experiments.

as a whole even though it may be only a temporary condition for the individual. A resume of our data from January 19th to May 25th by weeks indicates that the incidence of deficiency is evidently lessening somewhat. This probably reflects the greater availability of fresh foods but does indicate that, as yet at least, the rationing program has not noticeably decreased the amount of riboflavin available.

SUMMARY

1. The riboflavin content of 658 urine specimens has been determined.

2. Evidence is presented that the riboflavin per milliliter of urine is a more constant value than others which have been used in expressing riboflavin excretion.

3. Saturation tests seldom give more information than can be obtained by analysis of a fasting morning specimen.

4. There is a widespread incidence of riboflavin deficiency in this area.

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